

Can Science be Relevant in Developing Countries?

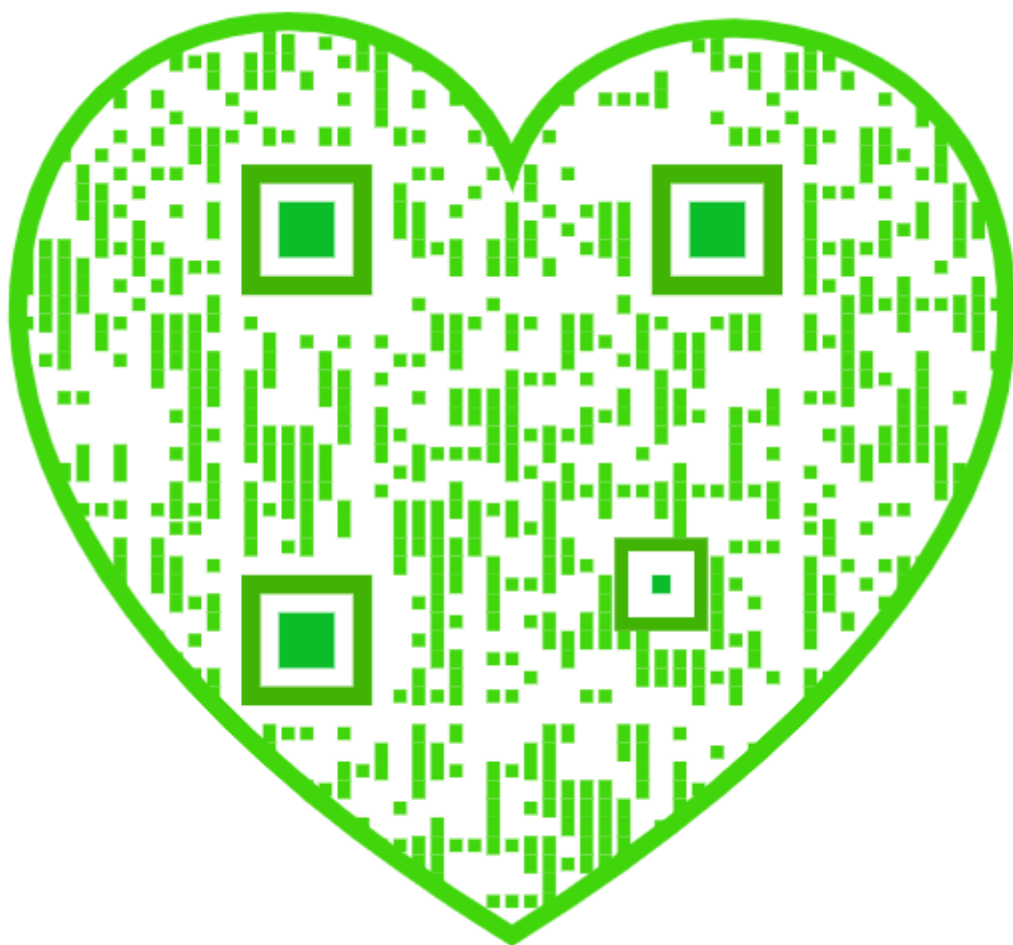
THE romantic and for that matter the liberal view of science includes the belief that scientific research and development are among the most valuable gifts that prosperous countries can bestow on those less fortunate. Developing countries themselves have usually not resisted this belief. The spectacular way in which the Indian Government has sponsored the development of nuclear energy as well as of a great many projects in basic physical science which compare excellently with similar activities elsewhere is at once a testament of faith and a mark of cleverness. To be sure, there are always those like Dr Hastings Banda, the President of Malawi, who are prepared to intervene with sceptical declarations that the immediate need in the smaller and poorer developing countries is for projects that will contribute to prosperity, or to health, or to the solution of social problems, in the immediate future, not the decades ahead. And there are times when, quite properly, people like Dr Banda are as clamant in their insistence on a quick return as any commercial company looking for some way of turning investment in research and development into commercial profit. Between the extremes of the beliefs that all science is in the long run enlightening and that the only returns worth having are quick returns, however, there remains the awkward problem of knowing just what should be the place of science and technology in the development of the developing countries, especially as a part of the programmes of foreign aid which flit from time to time across the agenda papers of institutions such as the House of Commons and the Senate of the United States.

The International Council of Scientific Unions, the Royal Society and Lord Blackett have done a valuable job in providing an umbrella for a study of the problem by Mr Graham Jones, a man with practical experience in the old British Commonwealth and elsewhere, of the problems which cry out for attention and of the steps which might be taken to solve them (*The Role of Science and Technology in Developing Countries*, published for International Council of Scientific Unions by Oxford University Press, £1.75). With a refreshing—even a daring—innocence, Mr Jones says that his object is to consider science and technology “as an instrument for economic growth”. He points out that even if it is permissible for people in advanced countries to discount the value of the GNP as an indicator of progress, poorer countries are bound to be preoccupied with economic growth, not necessarily as an end in itself “but as a means to other ends”.

So far, so good. And Mr Jones goes on to argue the importance of the linked development of industry and agriculture; the danger is that developing countries may find a handful of workers in factories making advanced products so as to support much larger numbers of unemployed. In the same spirit, Mr Jones points out that technical innovation as such is usually less important to

developing countries than the capital investment which is necessary to exploit the technology that exists already. He argues that developing countries should strive for some balance between basic and applied research, but he does acknowledge—which is proper—that in a country which may be anxious to put its universities in order, basic research may loom large for a time at least. Mr Jones says flatly that in developing countries, the mechanisms of market economics must be supplemented by “science planning”, by which he means that the governments of developing countries must be prepared to determine technical objectives and then provide the resources to attain them. What this implies in practice is that a nation at the beginning of its development may be well within its rights to deny the right to possess motor cars to its subjects for the sake, perhaps, of more investment in agriculture. The difficulty here, of course, is that there is a danger that in trying to anticipate the course of history so as to leapfrog ahead, the governments of developing countries may make the wrong choices and leap backwards instead. Mr Jones offers them the sensible prescription that the best choices are those which save capital and which are at once efficient and labour-intensive. But even this is not a sufficiently tight specification to be much help. On scientific education in developing countries, Mr Jones is sensibly pragmatic—let there be science teaching of some kind as soon as possible, let secondary and higher education be broad rather than specialized, but do not entirely eschew the advantages which come from having a handful of people in a country who can rub shoulders with colleagues of international standing from elsewhere. But in general his message is a little of a letdown: “aid and trade”, he says, “may be more helpful than research”.

This practical advice will no doubt help in important ways to inform the development of programmes for aid to developing countries in the years ahead, at least to the extent that developed nations deign to let their consciences worry them enough to take the trouble. Unfortunately there remains a string of questions that will not indefinitely be diverted by Mr Jones’s pragmatic commonsense. What, for example, is the place of advanced technology in slowly developing countries? By now, most people will be well aware that nuclear reactors may be decisively too advanced for some quite advanced countries, let alone their poorer neighbours on whom a great many research reactors have been wished in recent decades. But what role in economic advancement of developing nations might be played by less spectacular forms of technology? Although it may be hard to turn a nation of peasant farmers into a nation of transistor-makers in a few short years, may there not in the long run be something in Professor Arthur Lewis’s familiar argument that the developing nations of the tropics should be encouraged to emerge as industrial economies, letting temperate regions,



such as the prairies of North America, grow wheat? Further, is it not possible that the immense economic benefits which advanced nations derive from devices such as the telephone might yield even greater benefits in countries such as India? To be sure, the social consequences of introducing telephones into India on the scale on which they are now apparent in the United States could not be inferred from the experience of the United States. Moreover, it is clear that the capital cost of providing anything like a comprehensive telephone system in circumstances like these could create all kinds of economic problems. The truth remains that there is no natural law to demonstrate that the only way in which a country can develop is to follow quickly in the steps which advanced countries have taken. One of the important services which science and technology might provide is to show where corners might be cut. Intermediate technology, with its artesian wells and fertilizer sprays, is, of course, important, and who will deny that cheap plumbing is the best long term prophylactic of cholera? But there is a need at least for serious studies of the potentiality of moderately advanced technology.

Another perennial problem, dealt with repeatedly in Mr Jones's book, is to determine the extent to which research and development carried out in advanced countries can be applied elsewhere. On the face of things, and no doubt for the circumstances in which urgent benefits are looked for, there are limits to the extent to which a new technique developed for the purposes of an advanced society can be exploited in developing nations. A part of the trouble, of course, is that even the introduction of, say, a manifestly improved variety of cereal first requires the training of the farmers, and also of those who must train the farmers. Experience shows, however, that exhortation is not enough; practical experience is also necessary. But it is also at some stage important to break down the natural suspicion of populations at the receiving end of aid that they may be being fobbed off with the solutions to other people's problems. That said, however, there is a vast range of problems, especially in medicine and agriculture, where organized research on an international scale could easily bring benefits as great as any looked for by the idealists of the thirties. And is it not, in any case, at least a possibility that in some important fields of scientific effort of potential benefit to developing countries, recent events have seen a deterioration, not an advance? Is it, for example, possible that research in trypanosomiasis in Africa is now less well organized than a decade ago? To be sure, there are plenty of people killing tsetse flies, but is there not also a case for a more vigorously directed and more centrally organized programme of basic research? By the same test, the medical problems of many developing countries, especially those in the tropics, should be amenable to all kinds of internationally mounted projects. After all, the greatest success of the past few years has been the introduction into India, Pakistan and South-East Asia of strains of wheat and rice originally developed at plant breeding establishments in Mexico and the Philippines. It would be a great misfortune if the potential importance of opportunities like these was neglected by those who have the responsibility for designing programmes of aid to developing nations.

There remains the question of how valuable to developing nations is the liberal understanding of the natural

world which science and scientific research can provide. This is a perpetual source of argument. The Israeli government has in its time had to contend with arguments from articulate critics that even such an advanced developing nation should not waste resources on basic research. How much more difficult must it be for the governments of, say, tropical Africa to spend money on research and education in science when there are schools to build and drains to dig.

Fare Score

THE reason for worrying about the way in which international airlines fix their prices, well illustrated in the past two weeks by the traffic conference of the International Air Transport Association (IATA) at Montreal, is that aircraft are to many people the chief representatives of advanced technology which, by being mishandled, can be given a bad name. The chief preoccupation at Montreal has been the now ludicrous contrast between the standard transatlantic fare which the cartel—IATA is nothing else—has negotiated and the fares which enterprising travellers can pay for a flight across the Atlantic. By now, it seems to be established that charter flights can afford to charge as little as \$50 for a single flight between, say, London and New York. The airlines which belong to IATA are doing their best to compete by offering similar fares to the very young, to the very old, to those who are prepared to book several months in advance or even to those who are prepared to take a chance at airline ticket desks. One way and another, it is possible for those who have no great need to choose precisely when they arrive at their destination to cross the Atlantic for a third of the regular fare or even less. So by what logic can the director-general of IATA, Mr Knut Hammariskjold, have asked that governments should help his member airlines to beat off the competition of the charter airlines?

If the airlines need protection, it is not from their competitors but from themselves. In the past year, transatlantic capacity has increased by more than 17 per cent but the number of passengers buying tickets on the scheduled flights of IATA carriers has increased by only 0.9 per cent. The result is that on scheduled flights, the load factors have fallen sharply. Many airlines may be able to make good some of the ground thus lost by selling seats cheaply under new travel arrangements in the summer months, but there is no doubt that too many aircraft are chasing too few passengers. Unhappily, just as fishermen facing the prospect of declining stocks are tempted to increase the hunting capacity of their fishing fleets, so airlines hunting passengers are tempted to offer more and not fewer aircraft. The result is that a great proportion of the aircraft now crossing the Atlantic must do so uneconomically. At the same time, the people who fly by scheduled airlines rather than by charter flights or by non-IATA aircraft resent having to pay more than is economically justifiable. After all, the advantage of the larger aircraft now in service across the Atlantic is supposed to be not merely greater profit for the airlines but cheaper travel for consumers. Mr Hammariskjold and the airlines which he serves would be well advised to stop crying for government protection and simply reduce the prices that they charge.

... and also our Thanks to Mrs Smith

THERE is a disturbing tendency for acknowledgments at the end of even the most modest scientific report to assume progressively grander proportions. Is this a sad symptom of the insecurity felt by the poor scientist as he watches his grants contract and his colleagues become redundant? There must be some rational explanation for the effusions of gratitude which nowadays can fill up half a page or more of an otherwise sober manuscript.

The rationale for detailing the exact source of financial aid can usually be fashomed, if not appreciated by readers narrow-minded enough to be interested only in the scientific content of reports. Authors have clearly been driven to a state of mind in which they believe that it is simply a matter of no acknowledgment, no more money. And so Grant No. G536/41766XY is always lovingly recorded. But what of all those people who are regularly heaped with laurels for their assistance, technical and otherwise? Do authors live in fear of some sort of retribution if they do not extend grateful thanks to everybody concerned with their professional work? Will they arrive at the laboratory one morning to find their spectrophotometers and ultracentrifuges sabotaged because of some failure to acknowledge the titanic services of technicians and typists, not to mention the colleagues who happened to make a few intelligent remarks in the common room?

Some people, of course, are too important to ignore. Obviously it is worth flattering those marvellous volunteers who can fall asleep and wake up exactly when psychologists wish, who will supply blood at the prick of a hypodermic needle and provide daily samples of urine for months on end. Financial reward is clearly not enough, and they must bask in the reflected glory of the experimenter when he reports his findings. And, of course, other workers often find it useful to scan the last few lines of recent articles in their field to discover which hospital is likely to have a good supply of experimental blood or which college can provide suitable subjects for new and fiendishly complex visual tests. Without these selfless people much of the life sciences would clearly collapse. But why does nobody ever thank their rats, *Drosophila* and beagle dogs for submitting so willingly to surgery, sacrifice and other indignities? Doubtless somebody will rectify this injustice soon.

And what of technicians? Are they so badly paid that they must be thanked publicly for operating the instruments which they probably guard with their lives anyway? Or is it perhaps that they hold such tyrannical sway over laboratories that no more experiments could be performed if Rosemary Smith or Fred Brown were not thanked for their excellent technical assistance? The defiant author who calls their bluff and refuses to mention them will be worthy of an honourable mention in the annals of the history of science.

Much more sinister, however, seems to be the acknowledgment of professorial criticism and encouragement. "I thank Professor Blank for his constant support and for

suggesting the topic of this research", probably means that the professor induced the poor timorous author, on pain of dismissal from his team, to investigate some dismal problem which he, Professor Blank, found intriguing but had neither the time nor the inclination to tackle himself. His constant support and encouragement were no doubt required to keep the dispirited investigator at his bench. The professor's friendly criticism, if given at the wrong time, may eventually result in the ultimate indignity of a retraction, rounded off shamefacedly with "I thank Professor Blank for pointing out this error". What young scientist could ever recover from such a disgrace?

Some acknowledgments are blatantly political. Seekers after fossil man know that they may not be allowed near their precious sites again if obeisance is not done to their hosts. And palaeontologists, like geologists and some biologists, must always mention everybody who collected material for them; if they do not they may have to do the work themselves next time. As these contributors fill the final pages of their manuscripts with grateful thanks and grant numbers, they should reflect that acknowledgments consume each week almost a column of Friday *Nature*—the size of a small letter to the editor.

100 Years Ago



It savours of scholastic philosophy to speak of Nature as exercising any influence on the regeneration of races, and yet there may be sound philosophy in the old notion that when an individual or a class is in danger of being extinguished from want, Nature puts forward a special effort to preserve it. The sickly mother, the half-starved plant, is more likely to breed than the healthy and the vigorous. If we remove the peasant's family to the drawing room, it will cease to be composed of ten and twelve children. If we remove our daisies and cowslips to the greenhouse, their flowers grow double, and they ripen no seeds. The vine that has felt the frost is the one to pay the rent. Wherever we turn, in fact, we meet with examples of the universal law; and this law seems to be at issue with an important portion of Mr. Darwin's theory, namely, that in the struggle for existence, the vigorous, the hearty, and the well-to-do, elbow the weak and decrepid until they elbow them out of existence, and supplant them. If I have said anything above which can be construed into an impertinence, I unconditionally withdraw it. The only excuse for sorenness, is an impatience at what seems to the writer to be indefensible dogmatism. The days will not be ripe for scientific dogmatism until the Infallibility of Positive Philosophers has been generally accepted, and it does not do to forestal that millennium.

H. HOWORTH

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OLD WORLD

SCIENTIFIC SALARIES

Civil Service Pay Dispute

THE pay dispute between the British Government and the scientists who work for it has now gone to arbitration. Under the terms of the agreements which determine the procedures to be followed in the negotiation of salaries in the civil service, the outcome of the arbitration will be binding on both sides. It is, however, clear that the Institution of Professional Civil Servants, which represents scientists in the civil service, will be hard-pressed to restrain the anger of its members if arbitration does not lead to substantial increases of salary, especially for senior people. One complication is that of knowing how the dispute about pay will affect the moves which have already begun to unify the two branches of the scientific civil service—the experimental officer and the scientific officer classes—along the lines suggested by the Fulton committee on the civil service in 1968.

The chief cause of the anger expressed by members of the scientific civil service is that the government appears to have been tactless enough to offer no pay rise to those working in the senior grades. Principal Scientific Officers, for example, at present on an incremental scale rising from £2,820 a year to £3,902 a year would, under the government's proposal, receive no extra pay except that due to those still climbing up the pay ladder at £120 a year. The Institution of Professional Civil Servants had claimed on their behalf pay increases ranging from 7.8 per cent to 15.3 per cent according to status. In practice, the government has agreed to increase the salaries only of Scientific Officers, Senior Scientific Assistants, Assistant Experimental Officers and Scientific Assistants. The increases offered to those in these recruitment grades vary according to status within each grade but are usually a half or less of the amount which had been claimed by the Institution of Professional Civil Servants.

One of the most serious difficulties for outside students of the dispute is that the government's pay offer is based on a comparison of salaries inside and outside the civil service which has been carried out by the Civil Service Pay Research Unit and which is confidential to the parties in the dispute. The intervention of the Pay Research Unit is a consequence of the recommendation of the Priestley commission which argued in 1955 that civil service salaries should be determined by comparative methods, horizontally by comparison with salaries in industry, commerce and academic life, and vertically by the application of tests

of consistency within the civil service. What the Pay Research Unit has done is to compile statistics showing the relationship between salaries inside and outside the civil service, using data provided by several industrial companies to determine the pattern of industrial salaries. What the government has said is that, in effect, the more senior scientists in the civil service earn at present as much as they could expect to earn in industry.

The case against this interpretation, put forward by the IPCS in its meetings with the civil service department in May and June, is that the comparison between salaries in the scientific civil service and the outside world is in present circumstances invalid. First of all, the institution argues that even the Priestley commission acknowledged difficulties in relating the pay of the various grades of the scientific civil service to that of scientists outside. Second, the institution says that the practice has grown up among industrial companies of relating industrial salaries to those which can be earned in the civil service. Which means that neither scale can be considered an independent yardstick. At the same time, it is true, the institution argues that there are great differences between the ages at which people earn comparable salaries in industry and the civil service. If scientific salaries in the civil service are to be determined by any form of comparison, the institution would prefer comparison with the salaries earned by the other technically qualified classes in the civil service—engineers, lawyers, architects and the like—or even with those earned in the administrative class. For has not every committee on the subject—Barlow, Priestley and Fulton—spoken fondly of the importance of allowing good men to earn the highest salaries?

On balance, the civil servants' cause appears to be popular. Mr Airey Neave, the Conservative chairman of the House of Commons Select Committee on Science and Technology, went so far last week as to take Lord Jellicoe, the minister responsible for the Civil Service Department, to task for having let down scientists in the civil service. He considered the time has now come for a re-examination of the "long-term status" of the scientific classes in the civil service. This may be a far-reaching process, for it is now widely accepted that the present structure is anomalous, not merely because the pay of scientists is often less than that of engineers doing similar work but also because of the frequent overlap between different classes. The settlement recommended by the arbitrators, whatever it may be, will be backdated to January 1 this year but there is, of course, no way of backdating a revision of a person's status or classification.

NUCLEAR RESEARCH

Unattainable Centre

IT is nearly a decade since British nuclear physicists concluded that the next step in the development of nuclear structure accelerators would be a communal centre of research and not more money for individual universities to vie with each other. This admirable decision, if implemented, would have ensured that British nuclear structure research would remain something to be reckoned with in the late 1960s and 1970s.

What has in fact happened? So far, no final commitment has been made to build a national centre and the past ten years are strewn with a remarkable series of deferred decisions and political infighting, mostly about where the centre should be built—in the north of England at Daresbury, or in the south at the Rutherford Laboratory? The arguments put forward in favour of Daresbury are that the nuclear physicists at the Universities of Liverpool and Manchester would be left out in the cold if there were no further developments in the north. Arguments for the Rutherford site are based on the proximity of the equipment at Oxford and of the Atomic Energy Research Establishment at Harwell. Eventually a decision was made by the Science Research Council last year to site the facility at Daresbury, and in April 1971 a design study team was set up there under the direction of Dr R. G. P. Voss to look at the possibility of building the centre based on a Van de Graaff electrostatic accelerator.

Before commitment is made to the Van de Graaff project, however, there are some who think that the original aims of the National Centre of Research should be reconsidered in the light of the developments of the past decade. One question asked is whether the next step in accelerator technology which may have seemed obvious in the early 1960s is still the best next step. Should not the accelerators that will be in use in the late 1970s and early 1980s be given more consideration, for example? No doubt it would be easier to tell if some of the developments considered ten years ago had been built and exploited, but this has not happened, mainly because of lack of funds, not merely in Britain but elsewhere.

The result may be that British physicists may still be able to hold up their heads while seizing the option to install a tandem Van de Graaff generator that would produce 20 million volts. Such a development was the only one feasible ten years ago, for Van de Graaff electrostatic generators were the only accelerators then available to provide the energy resolution together

with the flexibility needed for nuclear structure research.

In the 1970s, however, the situation has changed. Recent developments in both cyclotrons and in superconducting linear accelerators must now be considered. Cyclotrons have an inherent advantage over tandem Van de Graaffs—there is no need to produce a negative ion for injection and no need to remove the electrons to form a positive ion for acceleration in the final stage. These processes are both inefficient and limit the intensity of the beam available from Van de Graaff accelerators. Moreover, developments in magnet design can now give cyclotron Van de Graaff hybrids energy resolutions that are comparable with those obtained with Van de Graaff accelerators alone.

A facility developed using two cyclotrons in series with a single Van de Graaff as interjector (in this case there is no need to form a negative ion) is nearing completion at Indiana, and the performance of this series of machines will be closely watched by British nuclear physicists. Similar developments are also planned at the Oak Ridge and Argonne National Laboratories.

Of more fundamental interest to future machine builders are present developments at Stanford University. The high-energy superconducting accelerator nearing completion there may also suggest how techniques can be developed to produce a flexible low energy accelerator for investigations of nuclear structure. There is no doubt that once the Stanford high energy machine is shown to be successful, there will be a surge of interest. Low-energy superconducting linear accelerators could well be plentiful in a few years.

Where do the British plans stand with respect to these new developments? It would indeed be remiss if cyclotrons and superconducting machines were not considered as serious alternatives to a Van de Graaff accelerator. The present design study team is asked to estimate the cost of a Van de Graaff project and its primary objective is to consider whether it would be best to build the accelerator or to buy it. Dr Voss is indeed aware of the alternatives to the Van de Graaff project and there is no doubt that they will be given serious consideration before any formal application is made for government money to build a National Centre.

STEEL RESEARCH

BISRA Unaffected

THE British Government's plans to infuse more private capital into the British steel industry are unlikely to affect Bisra—once the British Iron and

Steel Research Association but now the Corporate Laboratories of the British Steel Corporation. Indeed, Bisra may now be even more firmly entrenched as the corporation's central long term research organization. And the interest still shown by private companies (Bisra has nearly 300 associate members) means that the reorganization will have almost no effect on the research that Bisra undertakes.

The Corporate Laboratories under their new head, Dr Edward Calnan, appear to fit easily and logically into the overall research structure of the corporation, although inevitably at the expense of their autonomy. The head of Bisra is now but one member of the senior research committee of the British Steel Corporation which includes the heads of the research and development organizations of the corporation's product divisions (whose principal objectives are much shorter term research projects). Bisra spends £2.4 million a year, of which the British Steel Corporation provides £1.9 million—about 25 per cent of its total research and development budget. The rest of the income comes from private industry, sponsored work and royalties.

The new plans for the British Steel Corporation include the floating of several limited companies which will be wholly owned by the corporation in the first instance but into which private capital can eventually be injected, either through mergers or by the straightforward sale of shares. Two of the divisions of the corporation, Chemicals and Constructional Engineering, will probably be involved in their entirety, but the effects on Bisra are likely to be small. The Chemicals Division, for instance, is chiefly responsible for the production and marketing of the by-products of the steel industry—including tar, coke and gas—but its interests and those of Bisra seem to overlap very little. Bisra does, of course, possess considerable chemical expertise but this is chiefly concerned with such matters as reaction processes in blast furnaces and the prevention of corrosion. The situation is somewhat different where structural engineering is concerned, for Bisra does operate an extensive structural engineering service. A substantial proportion of the work is on a contract basis, however, and there seems no reason why a company in which the British Steel Corporation has an interest should not still make use of the facilities which Bisra has to offer.

The proposals to rationalize the overlap of Firth Brown Ltd and the corporation's Special Steels Division by what amounts to an exchange of assets could also have some effect on the refining research done by Bisra but, again, much of Bisra's work in this field is undertaken on a contract basis. Bisra clearly has unique experience of

refining methods and Firth Brown, already an associate member, will no doubt continue to avail itself of it.

CANCER RESEARCH

One New Broom

AFTER twenty years at Grosvenor Crescent, the British Empire Cancer Campaign for Research has a new home and a new name. The organization will be known as the Cancer Research Campaign (CRC) and the staff of 35 has recently moved into new premises at Carlton House Terrace. The campaign was forced out of its old home through the expiry of the lease, but a gift of £500,000 from the benefactor Mr Michael Sobell has enabled CRC, in partnership with the Royal College of Pathologists, to lease, restore and renovate its new apartments.

In line with its new home and new name, the report of the campaign for 1970, published this week, has a re-designed cover and outlines how the campaign's organization has been revised during the past year. The chief change is that the three principal activities of the campaign, the assessment of cancer research, finance and national appeals, will each be administered by a separate committee which will report to the executive committee. It is here that the campaign's policies are determined and put forward for approval to the Grand Council.

The CRC provides more money for cancer research than any other charity in Britain. The total income in 1970 from donations, legacies and interest was £2,496,889 of which £1,943,069 was spent on research projects. By comparison, the other principal cancer charity, the Imperial Cancer Research Fund, spent about £1 million, but this figure includes about £200,000 for the maintenance of its laboratories in Lincoln's Inn Fields. The CRC does not support much research in its own laboratories in the style of the ICRF, but it keeps strict control on the research it supports through its scientific committees. A notable example is the recently enlarged and renamed "Gray Laboratory of the Cancer Research Campaign", the radiobiology research unit at Mount Vernon Hospital.

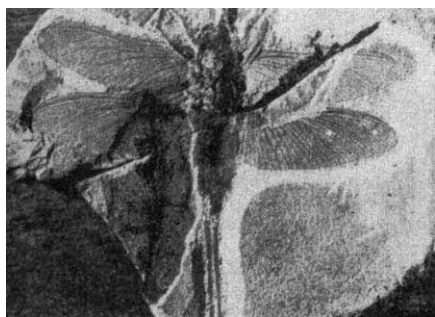
Most of the money provided in Britain for cancer research is raised through charity organizations. Of the £5.5 million total, some £3 million comes from CRC and ICRF and other smaller organizations such as Tenovus of Cardiff. The Medical Research Council in 1970–71 voted £2,166,000 and the remaining £0.5 million is covered chiefly by research in universities and hospitals funded by the University Grants Committee. Brigadier K. D. Gribbin, secretary general of the CRC, said last week that the campaign

had not, so far, turned down worthwhile projects through lack of funds—if more cash became available it would most usefully be used for strengthening the effort in fields of particular promise.

The chief danger to cancer research in Britain, as to all research at present, is inflation. The CRC's investment portfolio was below cost because of depressed prices at the time of audit, and £120,000 has had to be set aside from this year's surplus to guard against further losses. The Duke of Devonshire, vice-president of the CRC, said at the opening of the campaign's new apartments that unless income growth could be maintained above 10 per cent per annum over the next three years, "our research efforts in real terms cannot expand".

FOSSILS

Permian Change



PRELIMINARY reports from the summer 1970 expedition to the Urals of the Palaeontological Institute of the Academy of Sciences of the USSR indicate that the Palaeodyctioptera formerly thought to be predatory insects, were, in fact, herbivorous. Investigation of Middle-Upper Carboniferous and Permian strata has revealed numerous impressions of the seeds of gymnosperms bearing evidence of damage by insects. Associated with these finds (made on the banks of the river Chuna, a right tributary of the Podkamennaya Tunguska), there were found impressions of the wings, and parts of the bodies of insects, more than half of which belonged to the Palaeodyctioptera. The illustration shows a fossil of a new type of Palaeodyctioptera (*Permiakovia uralica*) found in Lower Permian deposits in the Urals.

According to A. G. Sharov, head of the expedition, these finds indicate that as early as 300 million years ago, there was a close ecological link between the Palaeodyctioptera and gymnosperms of Cordaites type. Previously, the Palaeodyctioptera were thought to be predatory. Sharov's finds, however, reveal that these insects had a strong sucking proboscis which penetrated the outer covering of the seeds and sucked out the fluid content.

SPACE FLIGHT

Death on Re-entry

from our Soviet Correspondent

THE death of the cosmonauts Dobrovolskii, Volkov and Patsaev during the re-entry of Soyuz-11 has inevitably evoked considerable speculation, not only about the cause of the tragedy, but also about what effect this will have on the future of the Soviet manned spacecraft programme.

Although the commission of inquiry into the disaster will not publish its report for some time, it would appear from a statement by Academician Boris Petrov, a leading figure in the construction of the Soyuz orbital station, that the death of the cosmonauts was due to a fault in the operation of the Soyuz-11 itself rather than to any radical defect in the design of the Soyuz type or to any insurmountable biological barrier.

Writing in *Pravda*, Academician Petrov points out that Soyuz craft have in the past performed a number of space missions, and that the cosmonauts have landed unharmed. "One can never rule out accident, however, when a complicated technology is being tested out and mastered".

He says little of the biological hazards of the mission. Medical and biological experiments, including radiation monitoring, were carried out, and will, he says, make an important contribution to various fields of knowledge. Significantly, perhaps, in view of the speculation on the possibility of long-term weightlessness being a contributory factor in the death of the cosmonauts, Petrov refers to weightlessness only as having been studied in connexion with its effect on the hydroponic garden aboard Salyut. Nothing is said about the success or otherwise of the "penguin suits" worn by the cosmonauts, which were intended to counteract physiological deterioration. However, his confidence in the continuation of the Salyut programme indicates that purely physiological causes are not considered to be the main cause of the tragedy. "We can say with certainty", he writes, "that the 'seventies will be an epoch of the development and wide scale use of long term piloted orbital stations with replacement crews, which will permit the changeover from episodic experiments in space to a regular watch of scientists and specialists in space laboratories". He does not indicate how long each tour of duty would last, but the general tone of his description of the type of experiments to be performed by such teams suggests weeks rather than days.

Petrov does not commit himself on the cause of the accident. Until

Academician Keldysh and his commission report, what can be gathered from the brief official announcements seems to indicate an apparently routine landing. Following the death during re-entry of Vladimir Komarov, in 1967, the re-entry systems of manned spacecraft were made fully automatic, and, as Petrov somewhat wryly comments, the present disaster is valuable evidence of the efficiency of the automatic soft-landing systems of the Soyuz craft. After the orientation of the Soyuz-11 for re-entry and the switching on of the braking motor, the final stages of its programme—aerodynamic braking, deployment of the parachutes and finally, "immediately" before landing, the switching on of the soft-landing motors—proceeded automatically. As far as the cosmonauts were concerned, loss of signal apparently occurred as for a routine re-entry. After the braking motors ceased to operate and aerodynamic braking was over, the signal was not re-established. Official sources stated simply that the cosmonauts were found "in their working places, without any sign of life". Until Keldysh's commission reports, it would seem, any further information on this subject must be largely speculative.

HISTORY OF MATHEMATICS

Committee at Work

from a Correspondent

THE proposal to form a national association for the history of mathematics was considered by a meeting in London on July 2 under the auspices of Dr J. G. Whitrow of Imperial College and Dr J. M. Dubbey of Thames Polytechnic. The immediate outcome was the formation of an organizing committee consisting of Dr Margaret Baron of Stockwell College, Mr A. Morley of Nottingham College of Education and Dr J. D. North of the University of Oxford. The proposal has been encouraged, in part at least, by the existence of a sub-commission in the history of mathematics within the International Union for the History and Philosophy of Science.

The meeting on July 2 was only in part procedural. The short papers presented to the scientific part of the meeting reflected the dual character of the history of mathematics as a teaching subject and a research subject, but the meeting itself recognized that such a separation can distort. Elsewhere, some participants noted, and especially in Germany, with its annual international conference at Oberwolfach, the exchange of ideas in the subject has been fostered for some time. History of mathematics is a subject in its own right, with its own philosophical and

methodological difficulties, which require a wide range of technical and historical skills and judgment in their attempted resolution. These factors were brought out by Mr J. V. Pepper who emphasized the importance in this field of the historian's traditional need of a knowledge and understanding of the nature and reliability of his sources, as well as skill and tenacity in their location and interpretation. The historical relation between theory and practice in much creative work in mathematics was also mentioned, as were the dangers of looking with hindsight at the problems and methods of earlier mathematicians. Reference was made to the Thomas Harriot editorial committee, which has recently received support from the Royal Society and from the National Endowment for the Humanities in America. Dr I. Grattan-Guinness of Enfield College spoke on the problems of the location of source materials; he thought these could well form the subject of a useful course for students of the history of mathematics. His remarks on the finding problems in London libraries (and some abroad), particularly for old periodicals, were perhaps a little harsh, but reflected the practical difficulties. He also referred to the frequent lack of appreciation of the context in which many ideas were introduced, quoting an important example from Georg Cantor's work on Fourier series in which sets appeared.

Mr L. Rogers of Digby Stuart College, who spoke on the relation between the history and pedagogy of mathematics, was interested in the psychological problems of learning mathematics. He considered that there was a complementary relation between the historical and mental origins of mathematical knowledge which could illuminate the nature of mathematics, and that the origins of mathematics were basically empirical: new problems generated new mathematical ideas.

HARIOTUS REDIVIVUS

History of Science

THOSE who believe in ghosts can add a new recruit to the spectres at the Bank of England after the unveiling there on July 2 of a plaque to commemorate the Elizabethan scientist and explorer, Thomas Harriot. Harriot, who died exactly 350 years before, was buried in the City of London church of St Christopher-le-Stock and rested there until the end of the eighteenth century, when the Bank of England expanded on to the site of the church and had it demolished. What happened to Harriot's remains is not known; his spirit, at least, has now returned, embodied on the plaque in a florid Latin

inscription which first appeared on his original memorial in 1621.

Harriot is famed chiefly for *A Brief and True Report of the New Found Land of Virginia*, published in 1588, and giving one of the earliest first-hand accounts of North America, which he had explored as a member of Sir Walter Raleigh's expeditions. His scientific achievements are only beginning to win their due recognition, for little appeared in print apart from a posthumous mathematical volume, *Artis analyticae praxis*, compiled in 1631 by some of his friends. He appears to have been the first Englishman to use a telescope—in 1609, before even Galileo had published his discoveries—and from his observations produced the earliest known map of the Moon, as well as lengthy accounts of comets, sunspots and the moons of Jupiter.

The plaque at the bank was unveiled by Professor David Quinn, the historian

from the University of Liverpool who has had much to do with the recent revival of interest in Harriot. It was the account of North America which first aroused Quinn's interest, and led eventually to the founding, in 1967, of a Thomas Harriot Seminar, meeting annually in England or the United States where Professor John Shirley of Delaware has been leading research for some time. Quinn is also chairman of a committee dedicated to publishing a complete edition of Harriot's writings, many of which exist in manuscript in the British Museum, though some of the most interesting papers are in a collection at Petworth House in Sussex. The committee already has the enthusiasm of a group of potential editors and the interest of a publisher, and although additional financial backing would undoubtedly speed the process, all the signs are that the project is well on the way to fruition.

Miscellaneous Intelligence

THE scheme first launched in 1954 by the Nuffield Foundation and the Royal Society for conveying scientists from remote parts of the British Commonwealth to Britain and in the opposite direction has, in the past year, been extended a little by a grant of £7,000 a year from the Commonwealth Foundation. Altogether, the two foundations and the Royal Society have spent £21,500 a year on this work, but contributions from elsewhere in the Commonwealth remain meagre. In 1970, less than 25 per cent of the total came from governments outside Britain. In the same year, according to the seventeenth annual report of the Royal Society Commonwealth Bursaries Scheme, most proposals for exchanges of people were to or from the United Kingdom. Only nine out of 101 applications in 1970 concerned non-British scientists wishing to visit countries other than Britain, and only two of these were successful. One of these grants took an Indian chemist to Ottawa for twelve months. Another, more modestly, carried a botanist from New Zealand to Australia.

BRITISH universities appear to be increasingly hard-pressed to spend money on extra-mural adult education. According to the annual report of the Universities Council for Adult Education for 1969–70, the number of adult education courses offered increased by just over one per cent to 8,000 but the number of students enrolled decreased by 0.6 per cent to 183,000. Many universities appear to have written to the council to complain, as Keele did, that

"adult education has the smallest part of the national budget and the most vulnerable". What seems to have been happening is that lack of money has made it necessary to restrict the length of adult education or to skimp on teachers. There is at the same time a tendency for university extra-mural departments to spend more of their energy in organizing self-supporting courses which, by definition, are more geared to demand than to what the universities consider to be the need. In practice, however, many university extra-mural departments seem to be anxious to follow the Open University in exploiting local radio stations. For what it is worth, courses on scientific subjects account for merely sixteen per cent of all those offered by the universities. Social studies are at the top of the list. The visual arts and history are close seconds.

THE Tweed River Purification Board seems to have done very little to purify the Tweed in the period to December 31, 1970, covered by its Seventeenth Annual Report. The board, undoubtedly distinguished by the presence of three dukes among its members, says that the river and its tributaries were in good condition except in May and June last year, but that many fish were killed not by pollution but because the rivers in which they swam dried up altogether. The board classifies the results of its analyses in categories labelled with such names as "satisfactory", "borderline", "unsatisfactory" and "not classified". It adds that "no specific research was undertaken".

NEW WORLD

International Whaling Omission

THE brief history of the International Whaling Commission offers a remarkable and perhaps unique example of pure commercial greed mixed in roughly equal proportions with pure folly. Instead of conserving the world's whale stocks at numbers which would allow the maximum sustainable yield, both now and in the future, the commission, like a feckless trustee who squanders the capital as well as the interest, has allowed one species after another to be driven to the verge of extinction.

This shameful record stems primarily from the unrestrained greed of the whalers which the commission has been unable to temper to any significant degree. Founded in 1946, the commission has a constitution which allows it no powers of enforcement and gives any member nation the right of veto. The conservation measures repeatedly urged by the commission's scientific advisers have repeatedly been ignored because of the opposition of one or two nations, sometimes the Dutch, sometimes the Japanese, who were even more short-sighted than their fellows. Moreover, the rule of the principle of the lowest common denominator has been effectively concealed for many years because of the commission's continued practice of holding its annual meetings in camera.

The gravest crime that can be laid at the commission's door is its failure to protect the blue whale, the largest animal known to have existed on Earth. The world's population of blue whales suffered a precipitous decline in the years before the Second World War—in 1930 more than 30,000 were caught. But there were still sizable numbers left in 1949, when the commission came into operation. For the next three years, more than 5,000 blue whales were taken, but it was not until 1965 that the commission decided to declare the species protected. At present there are estimated to be between 100 and 1,000 blue whales still alive, but even the higher estimate is thought to be too small to allow the whales to find each other and breed in sufficient numbers to offset the natural mortality. In other words, the blue whale is functionally extinct.

Because whale quotas are set in terms of an overall measure known as the blue whale unit (a unit is equivalent to one blue whale, two fin whales or six sei whales), the whalers have been able to turn their attention to the fin whale when the blue whale started to

become extinct, and to the sei whale when the fin whale population declined. The fast declining sei whale and the sperm whale now bear the brunt of the whalers' activities, but smaller species and even dolphins are being hunted as well. The consequence of this policy is that total tonnage of all whales caught in Antarctic waters fell from 526,280 in the 1949–50 season to 1,069 in 1964–65. Blue whales constituted 33 per cent of the former figure, 0.1 per cent of the latter. In 1965, when there were no more blue whales to be found, the commission agreed to make it illegal to catch them. Population experts hired by the commission have estimated that if the species had been maintained at its optimum population of about 60,000, the blue whale could have supplied indefinitely a sustainable yield of 6,000 individuals a year, containing more than 100,000 tons of oil and nearly 190,000 tons of meat, or enough to provide a daily 6 ounce steak for a year for three million people. Similarly, stocks of fin whales, if managed properly, could have supplied an annual sustainable yield of 20,000 individuals, amounting to a million tons of whale products.

Public outcry at the commission's dereliction of duty has been slow to arise, but the powerful conservationist movements in the United States have now taken the matter up, and the actions taken by the United States government may encourage similar steps in other countries. Last year Walter J. Hickel, the outgoing Secretary of the Interior, ordered as almost his last act in public office the banning of all imports of whale products by the United States. Hickel also classified as endangered species—thus granting protection under the endangered species act—the blue whale, the fin, sei and sperm whales and the bowhead, humpback, right and gray whales.

Congress too has been concerned to protect whales from the commission. A bill introduced by Senator Fred Harris and Representative David Pryor calls for a ban on the killing of all ocean mammals, whales and seals included. More importantly perhaps, the Senate last week passed a resolution requesting the Secretary of State to call for a ten year international moratorium on the killing of all species of whales. The resolution, which constitutes another slap in the face for the International Whaling Commission, cites the biological interest and im-

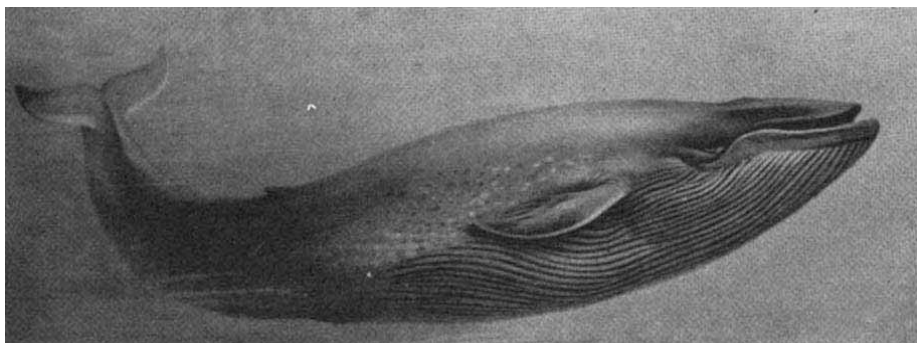
portance of whales, the driving of the blue whale to the verge of extinction, and continued killing of species that even the commission has declared to be totally protected.

The commission's roughest handling from the United States came during its annual meeting last month in Washington. U. Alexis Johnson, Under-Secretary of State for Political Affairs, told the commission at its opening session that public opinion round the world "clearly is growing impatient with what most people view as a failure by this commission to move quickly enough to prevent destruction of a unique natural resource". Johnson also directed the commission's attention to its failure to establish an international observer scheme, its continuance with the pernicious "blue whale unit" as a means of measuring catches, and its persistent setting of catch limits that exceed the sustainable yield.

The decisions reached by the commission at its conference this year do not differ greatly in kind from those that have characterized its previous deliberations, except on the question of international observers. The commission agreed to continue the use of the blue whale unit in the Antarctic for another year, although it did reduce the quota by 15 per cent, to 2,400 units. Judging by last season's catch, 38 per cent of which was sei whales (in terms of blue whale units), the new quota means that 5,500 sei whales will be killed, which is about nine per cent more than what the commission's own scientific committee estimates as the maximum sustainable yield for the coming season.

The commission's only unusual step was an agreement to make arrangements for member countries to place observers on factory ships and land stations of other nations. This important step would do the commission credit had it been taken when first proposed, in 1955. The commission actually reached agreement in principle

MOST of the articles in this section were written by Mr Nicholas Wade, Washington correspondent for the past year. Mr Wade has now left the staff of *Nature* and has been succeeded by Mr Colin Norman, from the London staff of *Nature*.



on a system of international inspection only eight years later, in 1963, but the scheme was not put into action during the next three whaling seasons and expired. (This was unfortunate because even species that the commission had declared protected continued to be taken, in error or otherwise. In 1967-68, for example, 66 blue whales were killed despite the protection they were supposed to enjoy.)

Not deterred by this setback, the commission continued its efforts to institute an observer scheme in its usual desultory fashion. The old 1963 scheme was affirmed again in 1967 by a working group, but at its 1968 meeting the commission decided to defer action for another year. The following year the commission took the bold step of recommending that its members "be requested to put into operation observer schemes as soon as possible". Nothing happened. Last year the Soviet commissioner recommended that the matter be discussed at the 1971 meeting in Washington. And at that meeting, after sixteen years of discussion, the member countries of the commission agreed to station observers on each other's ships.

Since the International Whaling Commission will survive no longer than the whales whose extinction it has supervised, which means that its days are probably numbered, a fitting tribute to its achievements is a chronicle of its efforts to save the blue whale. The following account is abridged from a thesis* by George L. Small, professor of geography at the City University of New York.

● In 1953 a special subcommittee of the Commission noted the alarming decline of blue whale stocks in what is known as Area II of the Antarctic and recommended that killing of blue whales in the area be stopped. The Dutch threatened to veto the proposal, so the committee recommended instead that the opening of the blue whale season be set back by two weeks. The only effect of this measure, which was agreed to, was that the whales had an

extra two weeks to get fatter before being killed.

● The scientific committee of the commission continued to press for conservation measures in 1954 and 1955 but these were again opposed by the Dutch delegation, whose science adviser did not believe that the blue whales were declining. The opening date was postponed another 10 days in 1955, and the commission also took the remarkable step of abolishing the Whale Sanctuary. This was established in the charter setting up the commission as an area of the Antarctic in which there were not many whales, which was why nobody was inconvenienced by it becoming a sanctuary. By 1955, however, the sanctuary contained many whales. The commission's scientific committee agreed to the sanctuary being opened with the hope of temporarily relieving pressure on other areas. But once having abolished the sanctuary, the commission was never able to re-establish it.

● By 1956 the catch of blue whales at South Georgia Island had declined from 3,700 thirty years ago to three. But the scientific committee of the commission opposed any measures to protect the blue whale because by this time the fin whale, on which the whaling industry now depended, was declining rapidly, and any effort to protect the blue whale would be at the expense of the fin whale.

● In 1957 the commission's scientists were still preoccupied with the fin whale and made no recommendations concerning the blue whale.

● In 1958 the Netherlands refused to accept any longer the prevailing quota of 14,500 blue whale units and successfully insisted that the quota be raised to 15,000.

● In 1959 the Netherlands left the commission, stated it would take up to 1,200 blue whale units regardless of season and succeeded in quadrupling its catch of blue whales. Since the unilateral action of the Dutch left the other nations at a disadvantage, the commission put back the opening of the season from January 7 to December 28.

● By 1960 the stocks of blue whales were so low that the commission proposed to advance the start of the opening season for blue whales to February 14. The Japanese objected. Then in order to entice the Netherlands back into the fold, the commission decided to abolish the quotas on whale catches for two years. The Dutch did not come back in, but more whales were killed that season than in any year since the Second World War.

● At its meeting in 1961, the commission had nothing to do in the way of establishing limits since there were no quotas to alter.

● The following year the Dutch came back and the scientific advisers to the commission recommended a complete ban on the killing of the blue whale. This time the Japanese objected. The commission itself, acting in plenary session, passed a motion that no increased protection should be given to the blue whale or any other species for another year. During that time some 1,000 blue whales, about 60 per cent of the existing population, were killed. According to Small, the commission's excuse for its inaction was the absence of a committee report that was not prepared for lack of funds. The cost of the committee's work was £8,000, the market equivalent of 4 blue whales.

● The next year a committee of population biologists appointed by the commission, known as the Committee of Three, warned that the blue whale had probably already been hunted beyond the point of recovery. A motion to ban the killing of blue whales throughout the Antarctic was resisted by Japan, and instead it was agreed that the species would not be hunted in the Antarctic except between 40° and 55° S latitude from 0° to 80° E longitude. The area kept open for hunting had furnished 75 per cent of the blue whales taken the previous season and also contained a species of whale referred to by the Japanese as "pygmy blue whales". Existence of this newly discovered species was doubted by non-Japanese cetologists, who believed that the animals were simply young blue whales.

● In 1964 a motion to close the "pygmy whale" area for the killing of blue whales was vetoed by the Japanese and the Russians. During the ensuing season the 15 floating factory expeditions in the Antarctic and their 172 catcher boats found only 20 blue whales.

● At its meeting in 1965 the International Whaling Commission agreed to accord complete protection to the blue whale throughout the Antarctic, for there were no whales left.

**The Blue Whale*. George L. Small. Columbia University Press, 1971. \$9.95.

AAAS

Scientists' Rights

by our Washington Correspondent

A HIGH level committee on safeguarding scientific freedom has been appointed by the American Association for the Advancement of Science. The committee will establish review procedures for cases in which scientific freedom has been infringed and will report on the "general conditions required for scientific freedom and responsibility".

The AAAS announced last December that it would set up a committee in response to a request from Senator Edmund S. Muskie that it investigate the case of two scientists employed by the Atomic Energy Commission, John W. Gofman and Arthur R. Tamplin. Gofman and Tamplin alleged that the AEC had cut their staffs in retaliation for their criticism of the present radiation standards.

But the AAAS committee is charged only with laying a general framework for this kind of investigation and will not consider the Gofman-Tamplin or any other individual cases. The AAAS may not be greatly disappointed at this outcome—in the absence of an established procedure the investigation of Gofman and Tamplin's complaints against the AEC could have been a delicate matter since the AEC's chairman, Glenn T. Seaborg, is the new president of the AAAS.

The AAAS committee is chaired by Allen V. Astin, former director of the National Bureau of Standards. Other members are Mary C. Bateson, professor of sociology at Northeastern University, Walter J. Hickel, former Secretary of the Interior, John H. Knowles, director of the Massachusetts General Hospital, and Earl Warren, former Chief Justice of the Supreme Court.

Astin, the chairman, has personal experience of the abuses the committee is investigating. In 1953, when director of the National Bureau of Standards, he was sacked by the then Secretary of Commerce after having criticized the efficacy of a battery additive known as AD-X2. With the help of protests from the scientific community, Astin was later reinstated.

Hickel, though not a scientist, is also aware of the hazards of speaking one's mind when in high position. He was fired from the Department of the Interior last Thanksgiving in retaliation for his public differences of opinion with the White House. Knowles too is no stranger to the way in which sectional interests operate in Washington; in 1969 his candidacy for the assistant secretaryship of the Department of Health, Education and Welfare is said

to have been blocked by the American Medical Association.

University scientists already have a defence against victimization in the existence of the American Association of University Professors, which appoints *ad hoc* committees to investigate individual complaints. The chief importance of the AAAS committee will be to devise procedures covering the more frequent instances of scientists in government agencies being persecuted for having spoken out of turn. Some four or five such cases have been checked out in detail by James S. Turner, a member of Ralph Nader's Center for the Study of Responsive Law. Turner, who plans to exchange ideas with the AAAS committee, is concerned with the way in which scientific information is often "diverted out" of the decision-making process in government agencies. "The scientific objectivity that is supposed to contribute to decisions is so often lacking that it is undermining the credibility of the regulatory agencies", Turner says. Information may be suppressed at many stages in the chain between the laboratory bench and the decision-maker at the top, leaving the individual scientist with the dilemma of whether to make his findings public and face the consequences.

SHUTTLE

Mondale's SST Flies

by our Washington Correspondent

FOR the second year in succession, Senator Walter F. Mondale has failed to block funds for NASA's shuttle, a vehicle which he looks upon with much the same proprietary interest as Senator William Proxmire regards the SST. Last week, by a 64-22 vote, the Senate authorized \$137 million for NASA to continue development of the re-usable space rocket. Undeterred by the size of their defeat, the Mondale forces point out that Proxmire campaigned for three years against the SST before he got anywhere; they are content at having exposed the holes in NASA's justifications of the shuttle programme, and will bide their time until NASA's budget comes before the Senate next year.

Mondale's aides attribute the strength of the pro-shuttle vote to the number of contracts, not yet placed, which NASA has in its gift; they claim that at least three senators who have voted against the shuttle before were lost for this reason. Nor do the anti-shuttlers enjoy the tremendous boost of the environmental sentiment which helped kill the supersonic transport. Nevertheless, Mondale's argument that the shuttle is a far grosser misallocation of

priorities than the SST—the greatest total cost NASA will at present admit to is \$12,800 million—may carry great weight in subsequent years when the shuttle's annual funding, at present comparatively modest, builds up to a more tempting target. Another factor in Mondale's defeat was probably the reluctance of most senators to add further to the unemployment in the aerospace industry.

The anti-shuttle movement was defeated last week in the House as well as the Senate, which was to be expected since the movement consists for the most part of Mrs Bella Abzug, a Representative from New York. What she lacked in allies, Mrs Abzug made up for in language. "The space shuttle is another camel's nose stuck under the tent of a three-ring circus," she told the House in introducing an amendment to delete funds for the shuttle from NASA's appropriations bill.

In the Senate debate on the shuttle, Mondale harped on arguments that can doubtless be dusted off and used again next year. Perhaps his most telling point was the fact that the total cost of the shuttle was estimated at \$9,000 million two years ago and at \$12,800 million in a study completed this year for NASA by the Mathematica Corporation. Citing the customary escalation of big hardware projects, Mondale predicted that the ultimate cost of the shuttle would be at least \$25,000 million.

Mondale also had fun with NASA's shifting justification for the shuttle—at first the shuttle was to service space stations from which men would be launched to Mars; now NASA has uncoupled the shuttle from the space station and claims that even if it carries only unmanned payloads into space the shuttle is economically justifiable. In an attempt to reduce the cost of the shuttle, NASA has recently decided to make the orbiter stage of the vehicle expendable. In response, Mondale quoted a statement by the Secretary of the Air Force, Robert C. Seamans, which seemed to put NASA out on a limb with this change of policy. "Present plans call for both the booster and orbital stages of the shuttle to be recoverable and hence re-usable", Seamans told the Senate committee on aeronautical and space sciences; "If we do not recover both stages, it's hard for me to see how the cost can be kept down."

For Mondale's opponents, his amendment to delete the shuttle funds from the budget was castigated as "a serious attempt to stop technological progress". Much play was also made about the Russian's success with the Salyut space station, which at the time of the debate was still inhabited by the three cosmonauts.

NEWS AND VIEWS

Can Geomagnetic Polarity Intervals be Classified?

THE problems of nomenclature in a rapidly progressing scientific field are three-fold. For one thing, any system of nomenclature adopted in the early days of a science should ideally be open ended in the sense that it should be able to accommodate new discoveries of fact without upheaval. In practice this is usually extremely difficult to achieve and in many cases is theoretically impossible. Second, it may well become apparent at some stage that a system of nomenclature which seemed rational and logical at the time it was proposed will seem less so in the light of more recent knowledge. This is more than merely a matter of accommodating new factual data. Apart from the simple case involving the naming of unrelated objects or events, a nomenclature is likely to carry within it certain assumptions about the way nature behaves—and it is unlikely that all these assumptions will prove to be valid in the light of subsequent discoveries. When new data have accumulated to the extent that a new, more natural scheme of classification can be perceived it becomes a case of having to revise the whole system of nomenclature. An increase in the quantity of data may well lead to a qualitative change in the theoretical framework within which the data fit. And, finally, there is the problem of human nature. Once a system of nomenclature has come into common usage it is not the easiest thing to overthrow.

All of these problems have arisen to a greater or lesser degree in the naming of geomagnetic polarity intervals. When Cox and Doell came to describe the first few well-dated polarity intervals within the range 0–4.5 million years, they immediately had to wrestle with the problem of open endedness. Simply to have numbered the intervals then known in sequence starting with the youngest would have led to a non-sequential series as soon as another magnetic interval was discovered; and it was clear, even at that time, that further discoveries were likely—an assumption which proved correct. It was equally evident, however, that the known magnetic intervals could be divided into two natural groups—one comprising intervals of the order of 10^6 years (which Cox and Doell termed “epochs”) and one comprising intervals of 10^5 years or less (“events”) which fell within epochs. The delights of this discovery were quickly apparent. The longer epochs were comparatively easy to delineate and were soon named after early workers in the fields of magnetism and palaeomagnetism (Brunhes, Matuyama, Gauss and Gilbert). The shorter events were then named after the sites from which came the first rocks in which the events were detected (Jaramillo, Olduvai, Réunion, and so on). On the face of it this system was perfectly open ended.

Unfortunately, it later became clear, both theoretically and experimentally, that the simple division into epochs and events was an illusion based upon incomplete data. In reality the lengths of magnetic intervals form a continuous spectrum from 10^7 years down to intervals too short to be resolved by potassium–argon dating. Clearly then the only system of nomenclature which is viable in the long term is one which assigns a different name to each magnetic interval, irrespective of its length. But by the same token such a system can only be devised when

every single interval has been discovered. In the light of experience it is obvious that to have adopted a perfectly open ended system right from the start was impossible (and is still impossible). Cox and Doell’s scheme, though adopted in a spirit of optimism which later proved unjustified, was nevertheless the best that could be devised under the circumstances. As a result, however, palaeomagnetists have inherited a scheme which is nonsense logically but which is likely to obtain for a long time to come.

So the first thing to be said about the Mesozoic and Tertiary polarity nomenclature proposed by McElhinny and Burek on page 98 of this issue of *Nature* is that it suffers from the same (and even more) disadvantages as the Cox and Doell scheme for the more limited period. To say as much is merely to state the inevitable; but it should also be said that McElhinny and Burek have made the best of what is, in the final analysis, an impossible job. What they have done is to analyse all reliable Tertiary and Mesozoic palaeomagnetic polarity data and thus show that a logical classification scheme is possible based essentially on reversal frequency. Thus they divide the last 280 million years of geological time into six intervals of 10^7 – 10^8 million years each (which they term magnetic intervals). Three of these intervals (230–200, 150–120 and 70–0 million years, respectively) are regarded as “mixed” in the sense that reversal frequency is so high that no predominant polarity can be discerned. The other three clearly have a predominant polarity but may contain much shorter “zones” of opposite polarity. Thus the reversed Kiaman interval (280–230 million years) has no zones, the newly named normal Graham interval (200–150) has three short reversed zones and the newly named normal Mercanton interval (120–70) has two reversed zones.

There is no doubt that this scheme will be of great practical value if only because it clarifies the existing state of knowledge and provides a formal framework into which new discoveries can, for the time being at least, be fitted. But ultimately it is likely to come to grief for the same reason that the original Cox and Doell scheme has come to grief—new discoveries will probably show that the picture is a great deal more complicated than current knowledge suggests. There must inevitably be a nagging feeling that McElhinny and Burek’s beautifully clear classification is the latest palaeomagnetic equivalent of deducing a linear law from two experimental points and that just as the distinctions between the epochs and events of the past 4.5 million years have become a meaningless blur so will the distinctions between the new intervals and zones. And because magnetic periods on the geological time scale cannot be defined with anything like the same precision as those of the last 4.5 million years, a good case can be made for suggesting that the disparity between the current picture and reality for the past 280 million years is greater than for the past 4.5.

Of course, none of this is to suggest that McElhinny and Burek have not performed a valuable piece of work but only that its limitations should be borne in mind constantly. The real danger—and there is an obvious precedent for it—is that once the McElhinny–Burek scheme becomes accepted it will be difficult to get rid of.

Ribosomes, Bound and Unbound

ONE way to explain the finding that some ribosomes are bound to the membranes of the endoplasmic reticulum of eukaryotic cells whereas others seem to be free in the cytoplasm is to suppose that the bound ribosomes are concerned with the synthesis of proteins which are destined to be secreted from the cell. Writing in the current issue of *Nature New Biology* (232, 8; 1971), Baglioni and his colleagues at the Massachusetts Institute of Technology report that membrane-bound polyribosomes are assembled by a process in which the large 60S ribosomal subunit first binds to the membrane, after which the small 40S subunit together with messenger RNA joins the complex. On page 102 of this issue of *Nature*, Williams and Rabin from University College, London, report their studies on the detachment from membranes of bound ribosomes by pharmacological reagents.

Their assay procedure is a devious method which depends on an enzymatic activity in endoplasmic reticulum that catalyses the rearrangement of disulphide bonds in proteins; the biological function of this enzyme is uncertain, but it may be concerned in ensuring the correct disulphide pairing and conformation of secreted and lysosomal proteins. When the correct pairing of the four disulphide bonds of the enzyme pancreatic ribonuclease is destroyed by reduction, the nuclease enzyme becomes unfolded and can be oxidized in such a way that the free -SH groups pair randomly. This version of the protein is not active, but the rearranging enzyme of endoplasmic reticulum can catalyse an interchange of the disulphide bonds of the nuclease to achieve thermodynamic equilibrium; this yields an active ribonuclease. Using this complex, and somewhat indirect, assay system, Williams and Rabin have previously found that the RNA content of microsomal preparations is inversely related to the rearrangement activity, and that addition of ribosomes in the presence of steroids inhibits all the apparent rearranging activity. Rabin and his colleagues have interpreted their observations to mean that when ribosomes bind to membranes they mask the activity of the "rearrangase" because the enzyme is located at the ribosome binding site. They have therefore used the activity of this enzyme—as measured by its effect on ribonuclease—to assay the extent of binding between ribosomes and membrane; it is this assay procedure on which they have based their results about how ribosomes bind to membranes and how steroid hormones and aflatoxin B influence this binding (see *Nature New Biology*, 230, 133 and 137; 1971).

It is reasonable to find that an enzyme, probably involved in the last stages of the biosynthesis of proteins for export, should be bound in some way to the ribosomes that make the proteins for export, and perhaps these ribosomes also make microsomal membrane proteins. Although, in the artificial assay system, the rearranging enzyme does not seem to work when the membranes are saturated with ribosomes, it is likely that in the living cell this enzyme is most active when the microsomal membrane is saturated with ribosomes. The significance of this paradox in the method is not clear.

A polycyclic carcinogenic poison, 0.12 mM aflatoxin B₁, decreases the quantity of bound RNA and increases the apparent "rearrangase" activity. Membranes treated with

EDTA to remove ribosomes can reattach them subsequently, but membranes treated with aflatoxin cannot bind ribosomes again presumably because they have been damaged irreversibly. Corticosterone (50 µg/ml.) but not hydrocortisone slows down the rate of ribosome loss from membranes caused by aflatoxin. Corticosterone, however, enhances binding and hydrocortisone inhibits binding of ribosomes to membranes treated with EDTA.

Oestradiol, pregnanediol and corticosterone enhance the binding of male ribosomes to male membranes, and testosterone enhances the binding of female ribosomes to female membranes. But male hormones in male systems and female hormones in female systems are without effect in this test. It is not apparent whether this effect results from the endogenous oestrogens in females saturating "oestrogen" sites, thereby only allowing the opposite sex hormone to have an effect in this system *in vitro*.

In the work described on page 102 some carcinogens are shown to unbind ribosomes from membranes in contrast to some non-carcinogenic analogues which do not show this property. Oestradiol protects against unbinding by the active compounds. Unlike EDTA unbinding, the carcinogens have an irreversible effect and prevent the binding of ribosomes to smooth membranes in the presence of oestradiol.

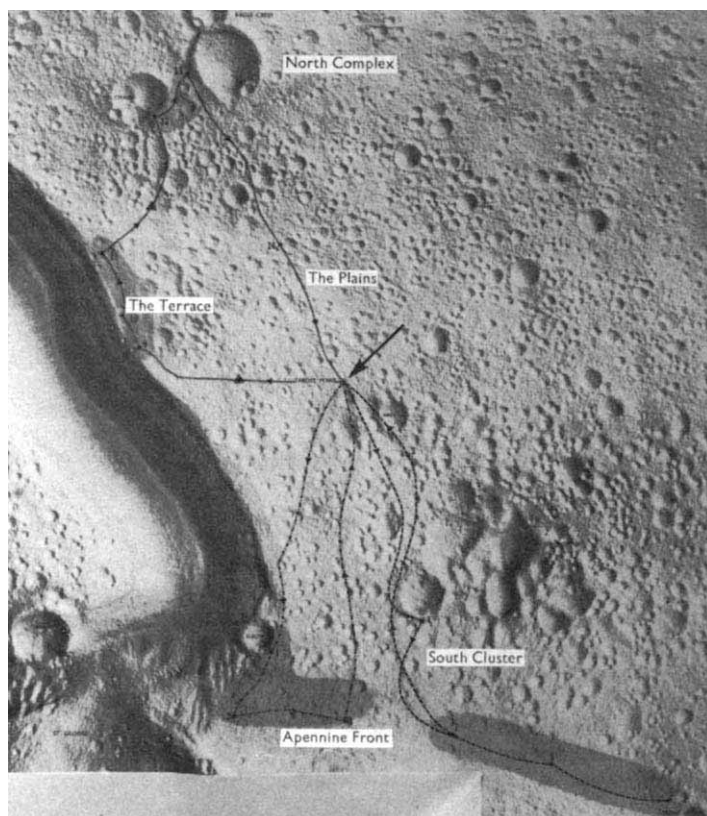
There are no experimental reasons to equate the effects observed in this complex system with the physiological effects of these hormones. The observations reveal some interesting aspects of steroid hormone behaviour; but perhaps not the only ones. One school of thought holds that the primary physiological effect of steroid hormones may be to regulate the synthesis of RNA in the cell nucleus. Williams and Rabin may perhaps be observing an unusual tissue specific effect. If uterine microsomal preparations do not respond to oestrogens it would be difficult to argue that this is nonetheless a prime mode of oestrogen action. It will be very interesting to see the behaviour in castrated male rat liver.

An alternative possibility is that the prime effect of these sex hormones is indeed in the cell nucleus; but, in addition, these compounds which have an anabolic character enhance the binding of ribosomes to microsomal membranes, with the result that the synthesis of microsomal membrane protein is increased and consequently the capacity of the cell to synthesize molecules and to grow is greater.

The relevance of the observations to carcinogenesis is both more intriguing and less certain. A correlation has been established between a few carcinogens and unbinding of ribosomes from membranes, but acute and chronic poisons remove ribosomes from microsomal membranes. The experiments reported are all short term experiments. A striking feature of carcinogenesis is that it commonly requires lengthy administration before an effect is observed. The partial unbinding of ribosomes by the carcinogens is irreversible. It will be informative to hear what effect a prolonged change in the ratio of bound to unbound ribosomes has on the reproductive behaviour of liver cells. If bound ribosomes participate in the synthesis of the glycoproteins of the cell surface, then irreversible interference with the proper functioning of bound ribosomes may result in altered cell surface properties.

SPACE

More Science from Apollo



Relief map, prepared by NASA, of the region around the Apollo 15 landing site (indicated by arrow) several kilometres to the east of Hadley Rille. The map marks the projected routes for three excursions to sample material from the Apennine Mountain region, to study the Rille itself, to collect material from the mare—possibly one of the youngest on the Moon—and from the north complex which may be volcanic, and to investigate the cluster of craters south-east of the landing site.

IN spite of financially imposed restraints, it looks as if the tail of the Apollo space programme will bring in the scientific data which so far have been a secondary objective of the manned Moon missions. The recent report that the Fra Mauro rocks obtained from the Apollo 14 landing site are 500 million years younger than expected (see *Nature*, **232**, 10; 1971) makes this an appropriate time to look forward to the next two missions and their scientific aims.

The next lunar flight projected is that of Apollo 15, which should be launched on July 26. Improvements to the Saturn launch vehicle have increased the payload which can be taken to the Moon, and NASA seems to have taken some note of the criticism that they are merely running a prestigious "taxi service". Innovations incorporated in Apollo 15 are aimed at extending the research opportunities available both in lunar orbit and on the Moon's surface, although the much vaunted Moon rover which the astronauts will drive on the Moon's surface could probably have been usefully replaced by more valuable scientific equipment.

Each of the next two Apollo craft will carry small (80 pound) satellites to be released into orbit around the Moon. The observations made by these satellites will include liaison with a large magnetometer which the Apollo 16 crew will set up on the lunar surface. If successful, this will fill one of the most important gaps left by the failure of the Apollo 13 mission. Comparisons of the flux at the lunar surface with satellite measurements of the solar wind can be expected to lead to further developments of the theory, put forward by C. P. Sonett and his colleagues, of the Moon's structure and its formation (see *Nature*, **230**, 359; 1971).

By doubling the number of passive seismic stations on the Moon to a total of four, the Apollo 15 and 16 missions will provide the means for accurate seismic surveys of the Moon, and it can be expected that once three stations have been established the active seismic experiments left on the Moon will be used to discharge explosive charges. In particular, these experiments will help to determine the thickness of the lunar soil layer formed by meteoroid impact, and the depth of the upland

basin fill in the old crater Descartes which is the target for Apollo 16.

Perhaps the most important of the other equipment to be left on the lunar surface are a far ultraviolet spectrometer, a cosmic ray detector and several solar wind experiments. Because the present economic climate makes it unlikely that any further experiments will be placed on the Moon by hand for a long time, this belated burst of scientific activity by NASA is welcome.

Apart from the scientific experiments, the Apollo 15 and 16 missions will be important for the straightforward study of the geography and geology of the Moon. The Apollo 15 landing site near the intriguing Hadley Rille is the first site to be visited which is situated far from the lunar equator, and, quite apart from the value of a close range inspection of the rille, the surface rocks and soil may show differences from those collected on earlier missions. Apollo 16 will, if all goes well, land in Descartes. This is another near equatorial landing site (16° south and 9° east of the centre of the near side of the Moon), and lies in the central lunar highlands at one of the highest regions of the Earth side hemisphere. Perhaps one of these two missions really will find the 4.5 thousand million year old rocks which do not seem to be present near Mare Imbrium.

MEMBRANES

Liquid Paraffin

from our Molecular Biology Correspondent

PHYSICAL chemists who have taken ship on the treacherous waters of biology with the resolve not to get their feet wet have sooner or later to steer an uncomfortable course between the Scylla of physical intractability and the Charybdis of biological irrelevance. It is more commonly the latter that claims them. Once in a while, however, the promised land comes in view—a physically defined problem, directly amenable to the methods available, with a real biological message. A case in point is the structure and properties of lipid bilayers, which, there now seems no doubt, have a direct bearing on the interior of biological membranes. Paraffin structure, once an esoteric branch of physical chemistry, is now molecular biology. Perhaps the most elegantly conceived and successful approach has been that of McConnell who has used his spin-label technique to obtain information of remarkable refinement about the nature of the bilayer in time and space.

Much of the argument about bilayer structure has devolved on the degree of orientation of the paraffin chains. McFarland and McConnell (*Proc. US*

Nat. Acad. Sci., **68**, 1274; 1971) have now made bilayers with phospholipids, in which the spin label is covalently attached at various points on the chain. The paramagnetic resonance signals from oriented sheets of such bilayers can then give the average orientation of the label group with respect to the plane of the bilayer, within the time scale of the resonance relaxation time. Thus, if the interchange between possible geometrical states occurs more rapidly than 10^8 times per second the observed orientation will be the time-averaged direction, which is simply perpendicular to the bilayer plane for a label with its vector along the paraffin chain. If it is slower, the method will give an average orientation in space rather than time. Geometrical states of such long persistence in a paraffin would imply a high degree of cooperativity of motion, with a large cluster of chains swinging together into a new orientation, because the time scale of torsional motion about single bonds in isolated chains is of course much too fast to be of great interest.

Orientation is in fact observed: a label placed next to the polar head group at the outer part of the bilayer shows that this part of the paraffin chain is inclined at 60° to the bilayer plane. Moving the label away from the head group, the apparent tilt diminishes, and near the methyl terminus at the centre of the bilayer, the chain is perpendicular to the plane. The tilt near the head group necessarily changes the packing density, and by an amount very similar to the difference between the density of a liquid and a solid paraffin. The middle of the sandwich then is in effect a liquid, but at the edges it behaves essentially as a layer of solid domains. McFarland and McConnell point out the mechanism that this structure suggests for cooperative processes: an amphiphilic molecule (paraffin-seeking at one end, polar at the other) thrust into such a bilayer will cause a local disturbance, which could bring about what they term a domino-effect, successive clusters of chains swinging from one bent state to another. By changing their tilt direction the paraffin chains can, moreover, create a cavity to receive such an amphiphile. The possible implications of this effect for transport and depolarization processes are numerous.

A related approach, which attempts to gather microscopic information about the properties of a paraffin cluster is that of Weber and his colleagues (Shinitzky *et al.*, *Biochemistry*, **10**, 2106; 1971). The rotational relaxation times of a molecule are related to the viscosity of its environment, and can, as all the world knows, be studied by measuring the depolarization of fluorescence. Shinitzky *et al.* have chosen

to study first the nature of the paraffinic centre of a micelle. They use aromatic molecules of appropriate excited state lifetimes, which are insoluble in water, and calibrate the viscosity dependence of their fluorescence depolarization with paraffin oils of known viscosity. These species are then introduced into micelles, and used to measure the internal viscosity. This turns out to be in the range expected for a liquid, though greater than that of a bulk liquid paraffin. The rotation is anisotropic, with widely different relaxation times for rotations in and out of the molecular plane. By reference to experiments in other media, it is shown that the anisotropy is inherent in the geometry of the fluorescent molecule, and that the paraffin of the micelle is wholly isotropic.

An interesting observation of Shinitzky *et al.* is that in mixed micelles of detergent with cetyl alcohol or cholesterol—which introduce additional hydrophilic groups into the surface—

the microscopic viscosity in the interior is strikingly increased. This is in nice accord with the evidence on the effects of cholesterol on the structure of membrane bilayers.

HOMING

The Mystery Deepens

from our Animal Behaviour Correspondent

THE fact that many pigeons are able to home rapidly and accurately when released considerable distances away from their lofts in unfamiliar territory means that they must be able to use some system of bicoordinate navigation, and cannot simply use landmarks. The basis for this remarkable navigational ability is, however, so far unknown. The only complete theory proposed to date is that they are able to obtain information from the Sun about their position relative to that of their home loft, a view

Precursors of Ribosomal RNA

ANY molecular biologist casting around for new fields to plough would be well advised to steer clear of the mechanism of ribosome biosynthesis in bacteria, for a glance at next Wednesday's *Nature New Biology* is enough to convince anybody that that particular plot is already heavily if not over cultivated. No less than four groups, a dozen workers in all, report essentially identical experiments which lead them to conclude that the 16S RNA of the small subunit of the *Escherichia coli* ribosome is produced by cleaving bases from both ends of a larger precursor RNA.

A vast accumulation of circumstantial evidence indicates that the 16S RNA of *E. coli* ribosomes is not a direct product of transcription, and earlier this year Dahlberg and Peacock isolated what seemed to be this precursor molecule which, from its electrophoretic mobility in polyacrylamide gels, they estimated is some 200 bases longer than the mature ribosomal RNA. To prove that this molecule is indeed a precursor of 16S RNA, Lowry and Dahlberg have compared fingerprints of the two molecules. These showed that the precursor is a minimum of ninety-four nucleotides longer than the mature product and contains fewer methylated bases. Moreover, the fingerprint patterns indicate that these additional bases occur at both 5' and 3' ends of the precursor. Lowry, collaborating with Nomura, has also apparently shown that ribosomes reconstructed with the precursor RNA are inactive, but immature ribosomal particles containing the precursor can be

isolated from cells. Presumably therefore the maturation of the precursor is essential for ribosomal function, and at least some steps in the maturation occur in ribosomes which are only partially assembled.

Like Lowry and Dahlberg, Fellner and his collaborators, who for the past several years have been doggedly analysing the base sequence of mature 16S ribosomal RNA, conclude that the precursor has additional bases at both ends and that it is submethylated. They, however, estimate their precursor to be only about forty bases longer than the mature RNA; it might therefore be an intermediate product of maturation. Brownlee and Cartwright find that their preparations of precursor consist of molecules more than fifty-five bases longer than the mature molecule and they point out that because the 5' base of the precursor does not carry a triphosphate group it may well not be the initial product of transcription. Finally, the Paces and their colleagues say that their precursor is some 10 per cent larger than the mature RNA and that during its maturation sequences are cleaved from both ends.

That 16S ribosomal RNA is produced by the cleavage of both ends of a larger precursor is plainly beyond dispute. The initial product of the transcription of the 16S ribosomal RNA gene, however, remains to be identified and characterized, for the molecules studied by these four groups are probably all intermediates between the gene transcript and the mature RNA.

espoused by G. V. T. Matthews and C. J. Pennycuik (C. J. Pennycuik, *J. Exp. Biol.*, **37**, 573; 1971; G. V. T. Matthews, *Bird Navigation*, second ed., Cambridge University Press, 1968).

The Sun navigation hypothesis implies that when a bird is transported away from its home loft and then released, it is able to use the Sun to tell it the extent and direction of its displacement, that is, to find out its latitude and longitude relative to home. Matthews has obtained certain evidence compatible with this idea, and experiments using operant conditioning techniques have suggested that the sensory capabilities of pigeons meet the demands that would be placed on them by such a theory.

The situation is considerably complicated, however, because other workers (notably K. Schmidt-Koenig) have obtained results which imply that the pigeons do not use the Sun for true bicoordinate navigation, but simply as a time-compensated "compass" to maintain a fixed direction. It is held that the birds do not depend on the Sun to decide on this direction (K. Schmidt-Koenig, in *Advances in the Study of Behaviour*, 217, Academic Press, 1965).

Much of the controversy has centred on "clock-shift" experiments in which birds are subjected to artificial light-dark cycles in an attempt to upset their internal clocks and hence to interfere with their navigational computations. C. Walcott and M. C. Michener (*J. Exp. Biol.*, **54**, 291; 1971) have now published the results of a major series of experiments designed to change the direction in which pigeons believe their home lofts to be by shifting their internal clocks by various amounts before releasing them.

Although Walcott and Michener's preliminary results seem to support Matthews's hypothesis, the full results argue strongly against the idea that pigeons use the Sun for navigation. They clock-shifted birds by amounts ranging from five minutes to six hours but found that no clock-shifted pigeon ever flew to or investigated a false home area. Slowing down the birds' internal clocks by adding deuterium oxide to their drinking water also had no effect on their ability to navigate to their home lofts.

Pigeons, like other animals, certainly use the Sun as an azimuthal compass reference, but Walcott and Michener found no evidence that they can use it for true navigation. The "map" of the homing bird is therefore as great a mystery as ever, the more so in the light of recent evidence that this does not depend on a view of any celestial body and can operate effectively even after many days' imprisonment away from the home loft.

NUCLEAR PHYSICS

CERN Storage Rings

IN the latest issue of *Physical Review Letters* (27, 68; 1971), the first experimental results obtained with the intersecting storage rings at CERN are reported. The results are the work of a team of scientists from three separate institutions—L. G. Ratner from the Argonne National Laboratory, R. J. Ellis and G. Vannini from the University of Bologna and B. A. Babcock, A. D. Krisch and J. B. Roberts from the University of Michigan at Ann Arbor.

At this stage the interest in the experiment lies as much in the performance of the intersecting storage rings (ISR) as in the actual results. The scientific community will eagerly look at the details to see whether the designed properties are produced under experimental conditions. In the experiment of Ratner and his colleagues, the ISRs were each filled by repetitive injection from the CERN proton synchrotron to an intensity of 2 A which corresponds to 4×10^{13} protons. The beams were then coasted for 6 to 12 h with a loss rate of between 0.05 per cent and 5 per cent per hour which was a function of

the tuning of the rings. During this time the vacuum was maintained at 5×10^{-11} Torr.

The reported experiment is a study of proton-proton inelastic scattering at energies that are equivalent to laboratory energies of 500 to 1,500 GeV. Cross-sections are measured for reactions of the type $p + p \rightarrow \pi^+ + \text{anything}$ and $p + p \rightarrow p + \text{anything}$. The results are a continuation of work carried out at lower energies by other workers and these first results at a higher energy do not show anything unexpected. The π^+ and p cross-sections both continue to drop off with energy according to $\exp(-3.5P_{\perp}^2)$, as they do at lower energies, where $P_{\perp}$ is the component of the momentum of the outgoing pion or proton in a direction perpendicular to the beam. Such a drop off with momentum is as suggested by cosmic ray experiments.

Thus the first experiment with the CERN intersecting storage rings has revealed nothing unexpected in the inelastic scattering cross-sections of $p + p$ reactions. The performance of the storage rings is gratifying and the publication of experimental results a few months after the first head-on collisions were obtained is a tribute to the CERN staff.

A Human C-type Virus

VIRUSES which under the electron microscope look like the animal and avian sarcoma and leukaemia viruses and have what is called a C-type particle structure have from time to time been seen in sections of human malignant tumours; moreover, virus with C-type morphology has been isolated from human milks. Obviously these observations are compatible with the suggestion that human RNA tumour viruses exist, but to prove this the candidate viruses must be isolated in large amounts so that they can be characterized biochemically, serologically and biologically. In next Wednesday's *Nature New Biology*, Priori, Dmochowski, Myers and Wilbur describe a source of what appears to be a human C-type virus and some of the antigenic properties of these particles.

Last summer Priori and her colleagues established a cell culture from the pleural effusions of an American child, with the American form of Burkitt's lymphoma, who subsequently died of a varicella infection. The cells in this culture, a mixture of aneuploid fibroblastic and epithelioid cells, began at the tenth passage in culture to bud off virus particles with a C-type morphology. With sufficient of this virus, Priori and her colleagues, in collaboration with Old's group at the Sloan Kettering Institute, set up a series

of serological tests to see if the new virus is antigenically related to any of the known animal RNA tumour viruses or the herpes viruses which are associated with the African form of Burkitt's lymphoma.

To date they have, disappointingly, drawn nothing but blanks. The human C-type virus does not apparently carry any of the group specific antigens of any of the known animal leukaemia viruses, neither does the human virus have the interspecific antigen common to all the animal leukaemia viruses. The human virus is not apparently related to herpes viruses or the avian leukaemia viruses, but the cells producing this human virus can be stained with fluorescein tagged sera from some patients with lymphomas or osteosarcomas. Such patients may therefore also be infected with this mysterious virus.

When producer cells are co-cultivated with human embryo cells, the yield of C-type virus is increased suggesting that the virus can infect human embryonic cells and replicate in them. Experiments are in progress to test this idea and to determine whether the virus can transform any of the cells it can infect; their outcome should either eliminate another red herring or show that Priori *et al.* have hit on a human tumour virus.

POGONOPHORA

Animals of the Abyss

THE Pogonophora—marine, tubicolous, worm-like animals—have the unusual distinction of having been completely overlooked as a distinct group by zoologists until 1955 when the phylum was established by the Russian, A. V. Ivanov. These strange animals live almost entirely buried in soft sediments and may well have been missed for so long because many, but not all, of the species seem to have a preference for the abyssal regions of the oceans. But now that oceanographic is so much more intensive and more widespread than in the past and sampling of deep sea regions is more feasible, it is not surprising that new species are turning up in benthic samples, though they are not nearly so common as other marine worms such as polychaetes.

A marine zoologist who has made the group her special study is Dr Eve C. Southward of the Marine Biological Laboratory, Plymouth. In a new report (*Smithson. Contrib. Zool.*, No. 88; 1971), she adds another three species—*Diplobrachia floridiensis*, *Suboglinoides caribbeanus* and *Siboglinum bayeri*—to a growing list of species recovered by teams from several American oceanographic laboratories from sampling stations along the continental margin of North America from Nova Scotia to Miami. A total of twenty-four species from depths ranging from 40 m to more than 5,000 m have so far been recovered by these teams.

Dr Southward suggests that these Pogonophora fall into three distinct zoogeographic groups: (1) deep water species; (2) shallow water northern species; and (3) shallow water southern species, with a few of intermediate distribution. The shallow water populations are those which inhabit the area between the edge of the continental shelf (40–80 m) and about 600 m, and the layer of overlap between the northern and southern shallow water groups seems, for most species, to be between 35° and 36° N. The largest zoogeographic group so far recovered, with eight species, is found in the southern shallow waters of the Florida-Hatteras slope.

Now that some sort of pattern is emerging of the distribution of these north-west Atlantic populations of Pogonophora, the interesting question arises as to how the different species are dispersed. The answer, according to Dr Southward, seems to lie in the currents of the area. The adult animal is buried in its tube in the bottom muds so that there is unlikely to be movement of any significance although it could be swept downslope by turbidity currents. Dispersal of the species on a wider scale could, however, take place

during its development. The embryo develops inside the tube of the female and then emerges at a stage when it can secrete its own tube. This stage is very light and has no adaptations for swimming up to the upper layers of water and it could quite easily be transported passively for some distance by bottom currents. This assumption, Dr Southward suggests, agrees in most cases with current flow in the area.

SLIME MOULDS

Perfectly in Step

from our Cell Biology Correspondent

IT is a sign of the times that the biology of the slime mould *Dictyostelium discoideum* has become a topic eminently eligible for discussion in the *Journal of Molecular Biology*. In part, of course, this is simply because people working with this fascinating organism, with its natural history behind them, are at a stage at which they can profitably ask questions about its molecular biochemistry. But there is another factor at work. Molecular biologists bored with *Escherichia coli* and its viruses are, in increasing numbers, turning their attention to the problems posed by multicellular organization and *Dictyostelium discoideum* seems to pro-

vide an ideal bridge from the unicellular to multicellular worlds. Part of its time this slime mould exists as a population of separate amoeboid cells and at other times these cells assemble themselves into a fruiting body, complete with stalk and spore producing cap, or a slug-like migratory form, the cells of which are interdependent. Anybody wishing to investigate the biochemistry of cellular differentiation could hardly ask for a more accommodating organism.

Sussman and his collaborators over the past several years have related the activity of the enzyme UDP-glucose pyrophosphorylase, which is involved in polysaccharide synthesis, to the state of differentiation of the slime mould. The synthesis of this enzyme and of another, UDP-galactose epimerase, depends on the commitment of the cells to differentiate into a multicellular form, and as Newell and Sussman (*J. Mol. Biol.*, 49, 627; 1970) reported last year the pattern of accumulation of these two enzymes correlates with the morphogenetic changes of differentiation. Pyrophosphorylase and epimerase rapidly accumulate during the formation of the fruiting body and then, when a particular stage is reached, partially or completely disappear. If, however, a slug-like form differentiates, accumulation of pyrophosphorylase is slow and the enzyme does not suddenly dis-

Understanding the Oxidation of PAN

STRONG carbon fibres can be produced from a variety of polymer fibres, including PAN (polyacrylonitrile) and various cellulose based materials; to keep the cost of the starting material as low as possible, textile fibres which are in bulk production are generally used. These are rarely simple polymers of a single molecule but are usually copolymers containing a proportion of a second molecule to plasticize the fibre and to allow it to be spun and handled more easily. A third constituent may also be present to provide a site on the polymer chain for a dye molecule, an obvious essential for the textile industry. Thus the complexity of the polymer which is used as the starting point for carbon fibre production is so great that it is difficult to study the chemical changes taking place during the initial "oxidation" stage.

For PAN based fibres this stage consists of a heat treatment in air at >200° C and is quite critical for the subsequent formation of a strong carbon fibre. It is interesting therefore that Standage and Matkowsky have studied the behaviour of a fibre made up almost entirely of PAN. They report, in next Monday's *Nature Physical Science*, that instead of the usual 7 to

8 per cent of methyl methacrylate their fibres contain only 0.5 per cent and apparently have no dye sites. In an effort to understand the part played by oxygen in the oxidation stage they heated their fibres in a vacuum and only subsequently exposed them to the air. Their immediate observation was that the fibres changed colour only from white to deep red while being heated *in vacuo*—the same fibres heated in air would turn black very quickly. The observation that the red fibres slowly turned black on later exposure to air, even at room temperature, suggests that a spontaneous reaction with oxygen or nitrogen was occurring. Analysis of the fibres after various times of exposure to the air showed that oxygen was being absorbed and this casts some light on the intermediate structure which must be formed during oxidation. The problem which remains is to relate this behaviour of a relatively pure material in a vacuum to the changes occurring in a more complex polymer when it is heated in air. This sort of approach, however, is one of the few ways of attacking the problem and is being actively pursued by several laboratories in the United Kingdom and in the United States.

appear, and no epimerase is made. If the slugs are caused to differentiate into fruiting bodies they rapidly make pyrophosphorylase until its activity reaches that characteristic of fruiting bodies. If, however, the slugs already have that level of pyrophosphorylase activity before they are induced to fruit, the synthesis of a further 300–400 units of pyrophosphorylase per mg of protein occurs, together with a round of synthesis of epimerase.

Newell, Longlands and Sussman (*J. Mol. Biol.*, **58**, 541; 1971) have now followed up these findings with a series of experiments which indicate that the regulation of synthesis of UDP-glucose pyrophosphorylase occurs at the levels of transcription and translation. After disaggregation, differentiating slime moulds reaggregate to their former stage of differentiation, but, irrespective of the amount of pyrophosphorylase present before they were disaggregated, the cells make the full quantum of this enzyme associated with differentiation. And although during undisturbed differentiation of the fruiting body synthesis of this enzyme is not inhibited by actinomycin, presumably because it involves the translation of stable pre-existent messengers, its synthesis in reaggregating cells is sensitive to this antibiotic and so presumably entails further transcription. Other proteins are, however, made by reaggregating cells in the presence of actinomycin which indicates that the cells are not greatly damaged and do not lose RNAs non-specifically after disaggregation. Further, Newell and his colleagues have shown that exposing the disaggregated cells to EDTA or keeping them separated prevents synthesis of this enzyme.

During the differentiation of this slime mould the synthesis of UDP-glucose pyrophosphorylase is therefore regulated at both the transcriptional and translational levels. Cells committed to differentiation apparently transcribe the enzyme's structural gene and accumulate its mRNA which is subsequently translated when the cells receive signals telling them they have reached the appropriate stage of differentiation. After disaggregation, the cells rapidly recapitulate their morphogenetic differentiation but, as far as pyrophosphorylase synthesis goes, disaggregation seems to wipe the slate clean so that reaggregation switches on again the programmed transcription of phosphorylase mRNA which is rapidly translated. To use the words of Newell *et al.*, the activity of UDP-glucose pyrophosphorylase and the morphogenetic changes associated with fruiting are maintained "perfectly in step". How such synchrony is established and maintained will no doubt prove to be a fascinating story.

HEALTH

Above the Tree Line

from a Correspondent

THREE hundred delegates from thirteen countries and from the World Health Organization assembled at the University of Oulu in Finland from June 21 to 24 for the second international symposium on circumpolar health, organized by the Nordic Council for Arctic Medical Research.

The University of Oulu is the northernmost university in the world, and is 2,670 metres north of the University of Alaska, Fairbanks, where the first symposium was held in 1967. Although this claim was contested on certain technical considerations by representatives of Fairbanks in a friendly spirit, there is no doubt that the new Medical School of the University of Oulu, still partly under construction, was a perfect setting for the second symposium, with Professor Ole Wasz-Höckert (University of Oulu) as president.

It is an indication of the change of attitude of governments in recognizing the importance of the northern coun-

tries, and of a growing awareness of the changing problems of the circumpolar peoples, that the second symposium was on a much larger scale than the first, and some two hundred contributions on a wide range of topics were presented.

On the opening day, social-economic problems and community planning and development in the Arctic and Subarctic were discussed. Although of growing political importance, the population is still very sparse. About 150,000 live today above the tree line, of whom perhaps 70,000 are "native peoples". The total population of areas north of 60° N lat. is 6–7 millions only, and the number of Finnish Lapps has fallen to about 3,000. The problems of water supply, pollution and sewage disposal in conditions of perennially or seasonally frozen ground were discussed by several speakers. About 20 per cent, or 12 million square miles of the land surface of the world, is underlaid by permafrost, or ground which has been at a temperature below 0° C for two or more years.

On subsequent days, the symposium divided into three parallel sessions, so that delegates were able to follow their

How Collagen is Made

AN article by Lazarides and Lukens in next Wednesday's *Nature New Biology* buries the hypothesis that the three polypeptide chains of the collagen molecule are synthesized as one continuous chain of 300,000 molecular weight, and are then broken up into three pieces. The native collagen molecule is a three-stranded rope consisting of two types of chain, α_1 and α_2 , differing in composition, and present in the ratio 2:1. Polysomes from chick embryonic tissue were fractionated on a sucrose gradient and fractions were incubated in a cell-free system to see which would produce collagen. To identify the collagen, Lazarides and Lukens took advantage of the unique feature of its composition—the presence of hydroxyproline. This amino-acid is, in fact, a product of the enzymatic modification of proline after completion of the chain. Because this cell-free system fails to modify the prolines, however, the tritium-labelled completed chains, released into the supernatant, were treated with collagen proline hydroxylase. The enzyme caused the appearance of tritiated hydroxyproline in the chain and the release of one atom of tritiated water for each proline so modified. Only the product from a heavy polysome fraction was found to be a substrate for the enzyme. The identity of the labelled product as collagen was confirmed by addition of carrier collagen

chains and fractionation by gel electrophoresis, when the radioactivity followed the collagen profile.

Intact tissue was also incubated with tritium-labelled proline, and the polysomes were again fractionated by size. Completion of synthesis and release was then allowed to ensue in the presence of proline labelled with carbon-14, and the product was hydroxylated as before. The concentration of the two isotopes demonstrates comparable rates of synthesis *in vitro* and *in vivo* and also shows that there is appreciable hydroxylation *in vitro* while the chains are still in the process of synthesis. Adding unlabelled α_1 and α_2 chains, and fractionating them on an ion-exchange column, it transpires that both types are synthesized by the same polysome fraction. Their ratio, as judged by radioactivity, is anomalous, however, being 1:1, rather than 2:1, though the authors do not commit themselves about the significance of this.

From the sedimentation coefficient of the polysome fraction active in collagen synthesis, it is deduced to contain twenty-three and fifty-six ribosomes. Now a chain of 100,000 is expected to require a messenger that will accommodate thirty ribosomes. It therefore seems to follow that the collagen messenger is a monocistronic species, and that, as in all other eukaryotic systems so far studied, one messenger specifies only one polypeptide chain.

own particular interest, or gain an insight into other areas, whether they were organization of public health care, disease and infection, genetics, nutrition, psychiatric problems, clothing and cold physiology, or odontology and ophthalmology. Comparison of race, and of rural or urban life, was a recurrent theme in many of these topics.

The value of the symposium was to derive benefit from the experience of different countries, and of civil and military authorities in various fields. As pointed out by Professor E. C. Albrecht (Jefferson Medical College, Philadelphia) this applies particularly to organization of health care. Other contributors discussed the progress achieved by the increased control of tuberculosis, a disease which was once widespread in the circumpolar regions. Mental health remains a problem, however, with a marked increase in depression and alcoholism.

In the field of clothing and protection, experimental and theoretical studies carried out on military clothing in the United States and the United Kingdom were discussed by Dr R. F. Goldman (US Army Research Institute of Environmental Medicine, Natick) and by Mr D. R. Gilling (Army Personnel Research Establishment, Aldershot) and Canadian developments were outlined by Mr G. T. Holmes (Department of National Defence, Ottawa). The British Army currently uses Canadian Arctic clothing.

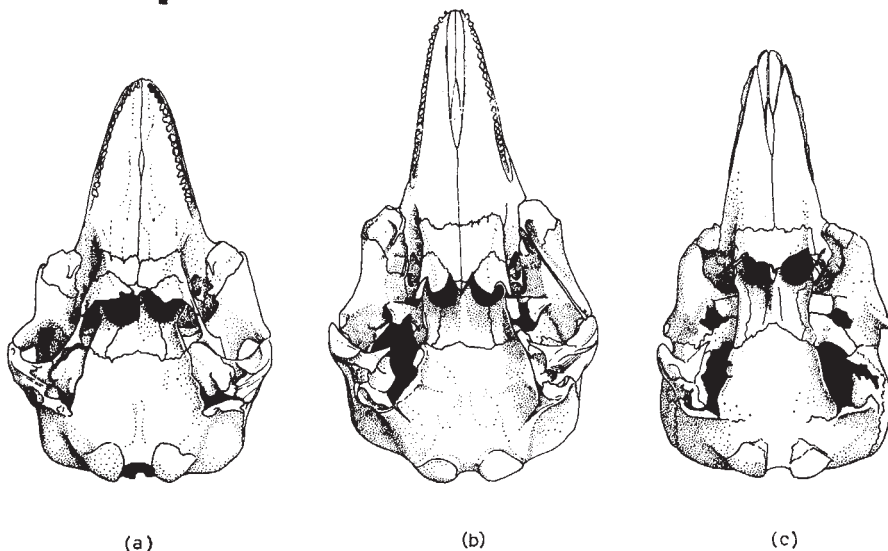
The role of the peripheral parts of the body was discussed both in relation to clothing comfort (Dr Z. Vokac *et al.*, Institute of Work Physiology, Oslo) and to adaptation to cold. Dr D. W. Rennie (State University of New York) described an interesting comparison between the Eskimo and the Korean diving woman, each showing adaptations to their own ways of life and work. The introduction of modern vehicles such as the skidoo for land transportation in the Arctic has led to increased cold stress to the face and hands, calling for further adaptation.

The history of treatment of frostbite was dramatically illustrated by a film with a commentary by Drs E. Eriksson and P. O. Granberg (Karolinska Sjukhuset, Stockholm) which emphasized the importance today of prophylaxis and conservative treatment, in contrast to the earlier practice of amputation.

The symposium was concerned chiefly with the negative effects of a harsh environment; it was therefore encouraging to hear from Dr B. Wedin (Swedish Research Institute of National Defence) of one recent positive result of cold exposure, in releasing radioactive strontium already deposited in the skeletons of mice.

MAMMALIA

Rare Porpoise from the Gulf of California



In 1958 a new species of harbour porpoise, *Phocoena sinus*, was described on the basis of a skull which had been collected on the north-west shore of Punta San Felipe, Baja California Norte (K. S. Norris and W. N. McFarland, *J. Mammal.*, **39**, 22; 1958). A few years later another porpoise skull and part of a postcranial skeleton were recovered close to the type locality of the new

species and these were presented to the British Museum (Natural History). After examining the material, B. A. Noble and F. C. Fraser of the museum write in the latest issue of the *Journal of Natural History* (**5**, 447; 1971) that it does indeed belong to the rare *P. sinus* (see (a) in illustration) and it can be distinguished from the related *P. phocoena* (b) and *P. spinipinnis* (c).

"Reverse Transcriptase" and Lymphocytes

ENZYMES capable of using a synthetic DNA:RNA hybrid molecule such as poly dT.rA as template for the incorporation of deoxyribonucleotides have in the past year been detected in a variety of apparently normal as well as malignant cells including lymphocytes. And normal lymphocytes have been found to contain only about one-third the activity of acute and chronic leukaemic cells. Why do the malignant cells contain more of this activity than their normal counterparts? According to Penner, Cohen and Loeb, writing in next Wednesday's *Nature New Biology*, this difference merely reflects the fact that normal lymphocytes are quiescent cells whereas leukaemic lymphocytes are metabolically active and proliferating.

Penner and his colleagues reach this conclusion from a series of experiments in which they measured the amount of poly dT.rA dependent DNA polymerase activity in normal lymphocytes before, during and after the activation of the cells by the mitogen phytohaemagglutinin (PHA) which induces them to proliferate. Both stimulated and unstimulated lymphocytes contain an activity which can utilize poly dT.rA as template, and after stimulation the amount of this activity in normal

lymphocytes reaches the same level as that in leukaemic cells. All lymphocytes also contain another DNA polymerase which uses double-stranded DNAs as template.

During stimulation with PHA the five to ten fold increase in poly dT.rA dependent polymerase activity precedes the twenty-five to fifty fold increase in DNA dependent activity. Further, these two activities have different cellular locations and different sensitivities to heat denaturation; the poly dT.rA activity is the more sensitive of the two. There seems little doubt therefore that the two activities are distinct, but the question of the natural template and function of the poly dT.rA activity remains unresolved—it does not transcribe to any appreciable extent yeast RNA or the synthetic RNA poly rA.rU. Because *Escherichia coli* DNA polymerase I (the Kornberg enzyme), for example, can use poly rA.rU and natural RNA as a template it is still conceivable that the natural template for the lymphocyte DNA polymerase which uses poly dT.rA is DNA rather than RNA. Whether therefore this activity is a true reverse transcriptase capable of transcribing DNA from a natural single-stranded RNA remains open to question.

Contrast Illusions in Perspective

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The same sort of phenomenon that makes a large object seem lighter than a small object can be linked with the evolution of perspective in painting.

IN the house of the early Cubist painters in Paris lived a certain Maurice Princet, a somewhat derelict figure known to the group as *Le Mathématicien*. Princet is said to have addressed the following question to Picasso and Braque¹: "You represent by means of a trapezoid a table as you see it transformed by perspective, but what would happen if the fancy struck you to express the table as a type? It would be necessary for you to place it on the plane of the canvas, and return from the trapezoid to the true rectangle."

But had he tried this formula himself, Princet, the mathematician, might have been surprised at the result. Fig. 1 shows a table, not "transformed by perspective" so that its top is a trapezoid, but rather "expressed as a type" so that its top is a perfect parallelogram. Curiously, it does not look like a parallelogram, but like a trapezoid. We are subject to a visual illusion.

The conditions responsible are easily discovered. Removing the legs from the table makes the illusion almost disappear; placing on it a vase of flowers makes it become more obvious still. The error we make in judging the shape of the parallelogram depends on our seeing it as a table-top, with the implication that it represents a receding horizontal surface. It so happens that whenever two equal lines are convincingly depicted in such a way that one appears more distant than the other, the further line looks longer than the nearer. But why should apparent depth have such an influence on perception of size?

When people make estimates of the value of sensory stimuli it is generally true that their estimate depends not only on the actual value of the stimulus but also on what they expect its value to be. Deviations from the expected value are exaggerated,

so that if the value of a stimulus is less than expected it is underestimated, and if it is greater it is overestimated—the greater the discrepancy, the greater the error. Roughly, if the stimulus has an actual value of x and an expected value of E , the subjective estimate is out by an amount proportional to $x - E$.

In this formulation, expected value has rather a special meaning. Other things being equal, the "expectation" is conditioned simply by the prevailing sensory context, and the expected value approximates the average value of similar stimuli in the local environment. In the presence of large stimuli the expected value is high and in the presence of small stimuli it is low. Hence a particular stimulus is judged to be smaller in the former context than the latter.

These so called "contrast effects" occur in all sensory modalities. A light of a particular brightness, say a torch bulb, is judged to be dimmer in a brightly lit room than in a dark one; an interval of a particular duration, say a second, is judged to be longer in the fast movement of a symphony than in the slow one; a fruit of a particular sweetness, say an orange, is judged to be sourer when eaten with a sweet drink than with a sour one. Less familiar examples occur in the perception of visual form. Figs. 2 and 3 show the influence of contrast on the size of a circle and the orientation of a line: a circle is judged to be smaller when surrounded by large circles than by small; a line is judged to slope away from another line which crosses it.

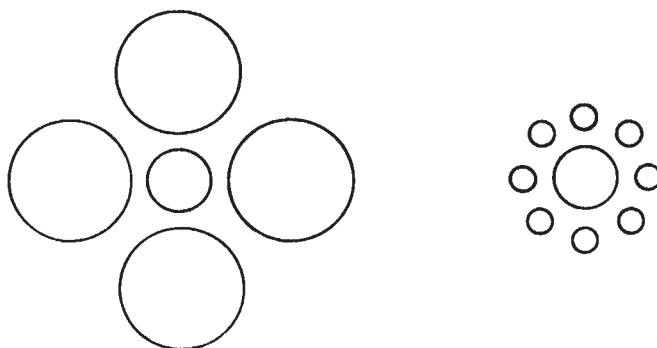


Fig. 2 A contrast illusion for size. The two central circles are equal in diameter.

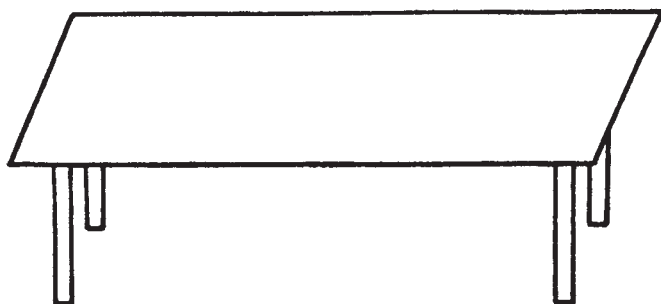


Fig. 1 For explanation see text.

The effect of a particular sensory context may persist to some extent when the context is removed, so that the subjective estimate of a particular stimulus is influenced not only by current but by previous exposure to stimuli of differing value. After wearing red glasses we see a piece of white paper to be tinged with green; after speeding along a motorway we judge a limit of 30 miles per hour to be dreadfully slow; after stepping off a heaving ship we feel the ground under our feet to be going up and down. Such after effects, too, occur for visual form. The effect for size perception can be shown

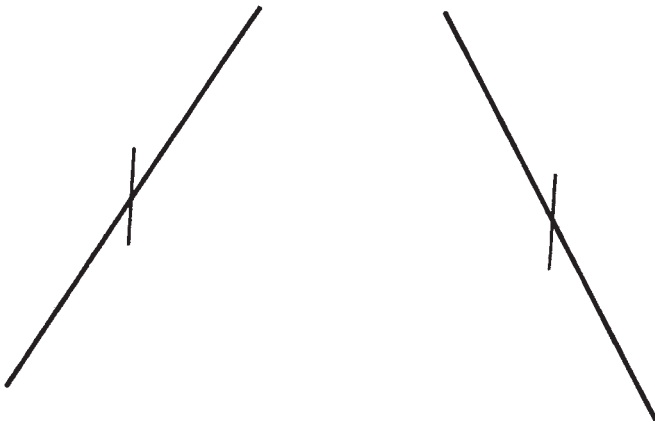


Fig. 3 A contrast illusion for orientation. The two short lines are parallel.

with Fig. 4 (ref. 2). Here, prolonged inspection of the left hand pair of gratings is used to influence our perception of the right hand pair: we come to expect the bars in the upper part of the field to be further apart than those in the lower and hence when we look at two gratings which are the same we judge the bars in the upper one to be closer together than those in the lower.

But the expected value is not determined exclusively by the value of similar stimuli in the environment. The expectation may be influenced also by established correlations which hold between stimuli in one dimension and those in another. A striking example, across sensory modalities, occurs in the "size-weight illusion". Here, an expectation based on visual information affects our judgment of a somesthetic stimulus. It is a reliable fact about the real world that large objects tend to be heavier than small ones, so that the size an object is seen to be is a potential guide to its weight and the expected weight of the larger of two objects is greater than that of the smaller. Hence if we are asked to lift two objects of different size but of the same actual weight, we judge the larger to weigh less

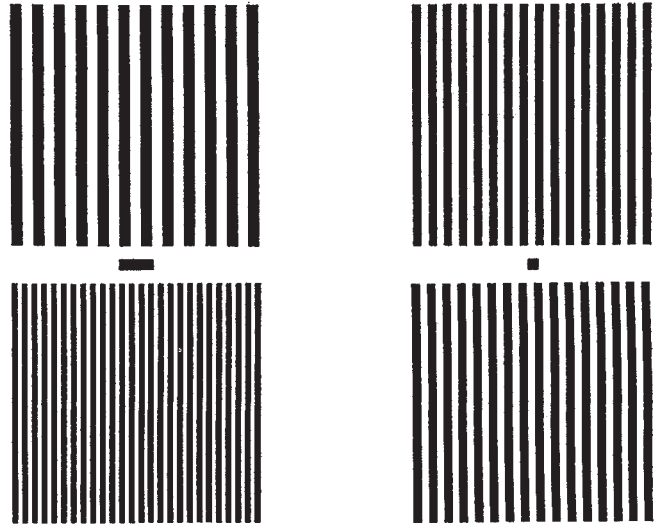


Fig. 4 An after-effect for size. The illusion is best obtained by placing the figure about 1 m away, and looking at the short horizontal bar between the gratings on the left for about 1 min before transferring the gaze to the equivalent spot on the right. (From ref. 2.)

than the smaller—we judge the pound of feathers to be lighter than the pound of lead.

The "depth-size illusion" of Fig. 1 is, to my mind, a phenomenon of this latter kind, where information from one stimulus dimension conditions our expectation about the value of stimuli in another. The nature of the optical projection to the eye is such that more distant objects on the whole give rise to smaller retinal images than near ones, and distance is therefore a guide to retinal size. The expected size of the further of two objects is less than that of the nearer, and hence if we see two lines which appear to be at different distances but have the same actual length, we judge the further to be longer than the nearer.

By this account, the depth-size illusion, the size-weight



Fig. 5 "Midsummer Rest under a Locust Tree"; northern Sung. (Peking Palace Museum.)

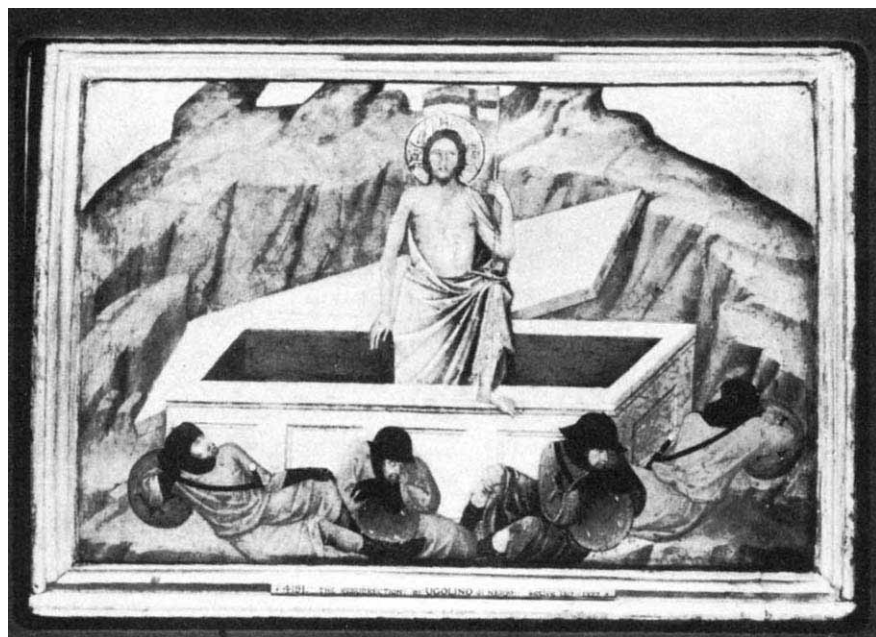


Fig. 6 "Resurrection" by Ugolino di Nerio (National Gallery).

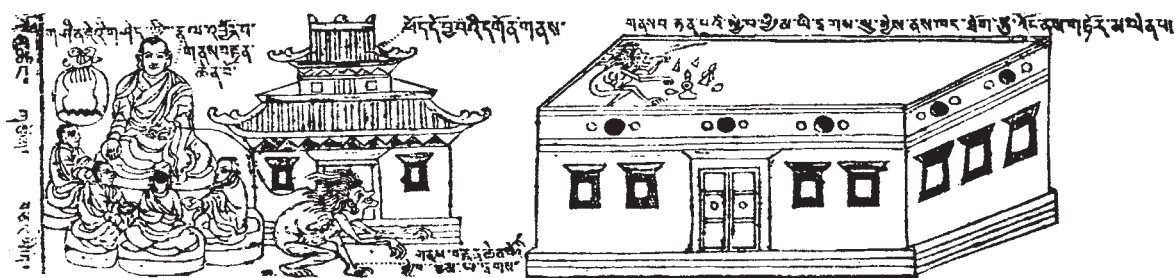


Fig. 7 Illustration from an eighteenth century Mongolian text.

illusion and the illusions shown in Figs. 2–4 are essentially similar phenomena, originating in the contrast between the actual and the expected values.

At a physiological level it is possible that these illusions have a common mechanism. The evidence of electrophysiology suggests that, for many stimulus dimensions, there are nerve units which respond preferentially to stimuli of certain values: any particular stimulus excites a range of such units, to a degree depending on how close their preferred value is to the stimulus's actual value, and the subjective estimate is based on an average of the overall response. The simplest explanation of the illusions is that those units which respond preferentially to the "expected value" are selectively depressed. In the case of the conventional contrast illusions, where the expected value is determined simply by the value of other similar stimuli in the environment, this selective depression would result from adaptation and mutual inhibition among those units excited by such similar stimuli. In the case of the illusions where the expected value is determined by stimuli in another stimulus dimension, the depression would have to involve some kind of cross-dimensional inhibitory interaction.

Whatever fascination these illusions have for scientists concerned with the mechanisms of perception, their importance in practice might seem to be small. To graphic artists, however, the depth-size illusion may cause real trouble. In the painting called "Midsummer Rest under a Locust Tree" from the Peking Palace Museum (Fig. 5) the tenth century Sung artist has drawn the couch on which the philosopher is lying as a perfect parallelogram, with the result that the back in fact looks longer than the front. Again, in the painting of the

"Resurrection" from the National Gallery in London (Fig. 6) the Italian Primitive, Ugolino, has done the same with Christ's sarcophagus, and the illusion is present once more. Rather than using the laws of perspective, these artists may well have implicitly followed Princet's suggestion and drawn their material as true to a type instead of true to what they saw: the two sides of a couch, the two sides of a sarcophagus, are equal in reality—and they have been made equal on the page. We may guess that they were puzzled by the distortion which they found, but it would have been in keeping with mediaeval practice to prefer a formal rule to the mundane evidence of the senses.

The same cannot be said, however, of the Mongolian artist who drew the temple in Fig. 7 to illustrate an eighteenth century Bhuddist text. He has drawn the far edge of the temple, which must have been meant to be square, not the same length as, but actually considerably longer than, the near edge. This way of doing things has seemingly no obvious rationale. But we can speculate about its history. What if this artist had innocently modelled his style on examples from the Sung school? He would have been tricked by his eyes into thinking that the Sung painter represented the more distant of two equal lengths as longer than the nearer. What was good enough for the Chinese would have been good enough for him, and in attempting to emulate them he has in fact given concrete expression to what was, unknown to him, the depth-size illusion.

¹ Quoted in Waddington, C. H., *Behind Appearance* (Edinburgh University Press, 1969).

² Blakemore, C., and Sutton, P., *Science*, 166, 245 (1969).

Carbon Compounds in Apollo 12 Lunar Samples

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Analysis of surface fines and core samples obtained on the Apollo 12 mission reveals small quantities of carbon compounds. The results from pyrolysis, mass spectrometry, ion exchange chromatography, and both optical and electron microscopy may shed some light on the origin and history of these organic molecules.

Small quantities of carbon compounds have been found in three samples of surface fines (12001, 34, 12033, 8 and 12042, 13), in one sample from the bottom of a 15 cm deep trench dug by the astronauts (12023, 8), in two core samples from 9 and 39 cm below the lunar surface (12025, 60 and 12028, 207, respectively) and in the interior of a lunar rock (12022, 81). The locations where these samples were collected, the site of the lunar descent module, and the basic topography of the collection area are shown in Fig. 1. We identified hydrocarbons (in the parts per billion range, except CH_4) using a pyrolysis instrument containing a helium atmosphere at 700°C , connected to a tandem gas chromatograph-mass spectrometer. The gaseous components were analysed by vacuum pyrolysis at 681°C and $1,084^\circ\text{C}$. Some gaseous components were separated with high resolution mass spectrometry. A search for amino-acids was carried out using Soxhlet extraction with hot water and ion exchange chromatography. In addition, an extensive examination with transmitted light and scanning electron microscopy was made in an attempt to establish the locales of the organic molecules and to shed some light on their

possible origin(s) and history. It is difficult to interpret the results unequivocally, which is not surprising in view of the difficulties encountered with the explanations of the Apollo 11 and 12 findings. This is illustrated by the apparent disparity in age of lunar fines and rocks^{1,2}.

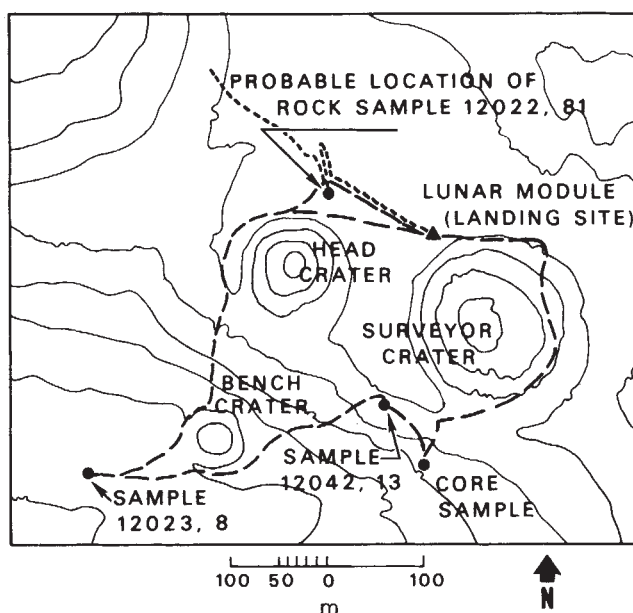


Fig. 1 Location of samples referred to in Figs. 2 and 3. Sample 12033, 8 was collected north of Head Crater on the second Extra Vehicular Traverse. The location of sample 12001, 34 was not accurately noted by NASA, although it was indicated that it was collected near the landing module (modified from ref. 10). ---, First Eva traverse; -.-, second Eva traverse; —, elevation contour lines at 5 feet intervals.

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Vacuum Pyrolysis

Lunar samples were pyrolysed *in vacuo* in a quartz tube designed to fit the gas inlet system of the mass spectrometer. This system was modified so that all evolved gases could be directed into the ion source of the mass spectrometer on reaching the desired pyrolysis temperatures. Before pyrolysis, the quartz tube containing the sample was degassed for 20 min by the mass spectrometer pumping system. A portable furnace was then placed around the quartz tube. The pyrolysis temperatures were kept constant during the mass spectral analyses.

Total carbon based on p.p.m. of carbon from CH₄, CO, and CO₂ was determined for Apollo 12 fines, core material and one rock chip. Before mass spectrometric determinations were made, calibration curves were calculated from various quantities of these gases. To prevent ambiguity with respect to the amount of CO in the lunar samples, we made high resolution determinations at $m/e=28$, to distinguish the relative intensities of CO, N₂, and C₂H₄.

Table 1 Vacuum Pyrolysis, Combined Results for 681° C and 1,084° C

Sample	p.p.m. C from CO, CO ₂ and CH ₄
12028, 207 (core 39 cm below lunar surface)	96.2
12025, 60 (core 9 cm below lunar surface)	111.2
12042, 13	140.8
12001, 34	115.2
12023, 8	109.2
12033, 8	108.6
12022, 81 (rock chip from interior of lunar rock)	89.6

All lunar samples were vacuum pyrolysed at 681° C. After pumping out the gases from the mass spectrometer, the samples were subsequently pyrolysed at 1,084° C and analysed as before. The contribution of carbon to the gases is shown in Table 1. The core samples and the fines showed CO₂ in almost equal quantities from the 681° C and 1,084° C pyrolysis. In addition, the total carbon from CO₂ was approximately the same in surface fines and in the two core samples. The rock chip, however, yielded 80% of the total CO₂ present when pyrolysed at 681° C and contained almost three times more CO₂ than any other sample. The CO content was roughly half of the amount found in all other samples. In all other samples pyrolysis at 1,084° C released 90% of the CO. CH₄ was produced in approximately equal amounts at both pyrolysis temperatures in all samples. The rock chip produced the most CH₄. Some of the CH₄ could be attributed to contamination, because the Ottawa quartz sand control from the LRL cabinet where the lunar samples were processed yielded about one-third the amount of CH₄ found in the lunar samples when pyrolysed under identical conditions.

All of the Apollo 12 lunar samples (except the rock chip) received in this laboratory were stored under nitrogen at the LRL. Pyrolysis at 681° C with high resolution determinations at $m/e=28$ showed a large amount of nitrogen for all samples. The nitrogen content was approximately 40% greater than the CO content at 681° C, even though the samples were placed under high vacuum for 20 min before heating. The subsequent pyrolysis at 1,084° C showed a considerable decrease in the nitrogen content to less than one-third that of CO.

Helium Pyrolysis

The lunar samples were pyrolysed in a stream of He at 700° C for 7.5 min in a modified Hamilton pyrolysis unit³⁻⁵. The He stream (3-5 ml./min) from the fused quartz pyrolysis tube was connected to a Perkin-Elmer model 226 gas chromatograph. It also served as the carrier gas for this unit. In turn the gas chromatograph was connected by means of a Watson-Biemann molecular separator to a Hitachi RMU-6E mass spectrometer.

A small trap, cooled with liquid nitrogen, was placed between the pyrolyser unit and the gas chromatograph so that, following pyrolysis, the trapped pyrolysis products could be introduced as a single sample into the gas chromatograph by means of a small portable oven which quickly heated the trap to 250°-300° C. The gas chromatograph contained a 50 feet × 0.02 inch internal diameter polyphenyl ether capillary column and a hydrogen flame detector.

Because it has been reported that similar aromatic hydrocarbons can be synthesized when methane is heated to 1,000° C over silica gel⁶, we passed methane over pre-pyrolysed lunar fines (12001, 34) at 700° C for 7.5 min. No evidence of any synthesis was observed (Fig. 3E). It could be argued that the initial pyrolysis, which removed the carbon compounds, destroyed any "catalytic" effect the fines might have had before heating. This argument is, however, tenuous because of the lack of experimental evidence. Another He pyrolysis control was run using a CO-H₂ (1/2.5, v/v) gas mixture over pre-pyrolysed lunar fines (12023, 8) at 700° C for 7.5 min, and did not show any of the hydrocarbons found in the lunar samples.

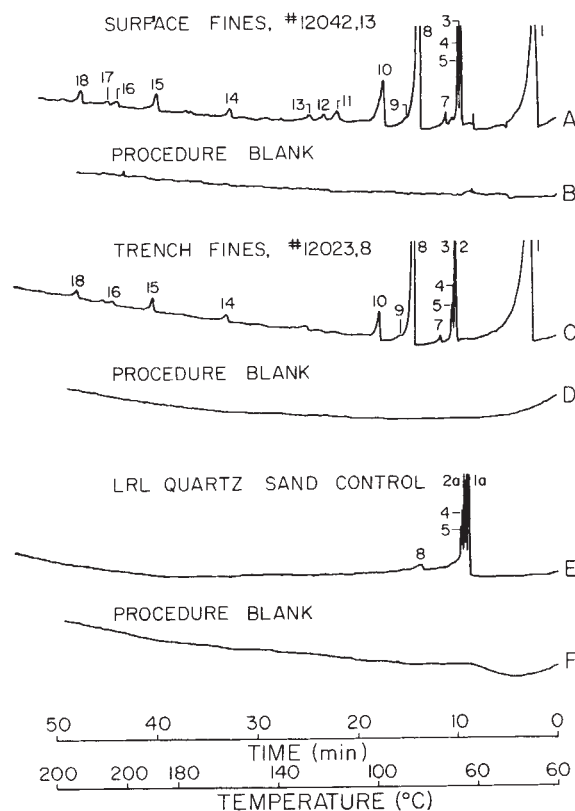


Fig. 2 Gas chromatograms of the pyrolysis products of *A*, surface lunar fines (sample 12042, 13), *C*, lunar trench fines (sample 12023, 8), and *E*, LRL Ottawa quartz sand control. Peak identities, all traces: 1, H₂, CH₄, CO; 2, C₂H₄, C₂H₆; 3, CO₂, C₃H₆; 4, C₄H₆; 5, C₄H₈; 6, C₅H₈ (small peak preceding peak 7 in trace *A* only); 7, C₅H₆; 8, benzene; 9, thiophene; 10, toluene; 11, C₂ alkyl benzene; 12, o-xylene; 13, styrene; 14, indene; 15, naphthalene; 16 and 17, methyl naphthalenes; 18, biphenyl. For trace *E* only: 1a, CH₄, C₃H₈; 2a, CO₂, C₂H₄. Additionally, on trace *A*: 1, also contains Ne; 2, C₂F₄; and on trace *C*: 2, CO₂. Compounds 9, 11 and 13-18 may well be artefacts synthesized in the pyrolyser as Nagy and Preti *et al.* pointed out^{12,13}.

He pyrolysis of lunar fines, the two core samples, and a rock chip (Figs. 2 and 3) showed the presence of primarily CO, CO₂, CH₄ and H₂. Many organic compounds were present in the p.p.b. range, together with lesser amounts of Ne and possibly COS. (See captions for Figs. 2 and 3.) It may be possible, however, that some of the higher molecular weight hydrocarbons

Table 2 Morphological Features and Differences Observed under Transmitted Light

Sample No.	Untreated lunar material	Pyrolysed at 700° C in He	Pyrolysed at 1,084° C in vacuum
12001, 34 fines (a)	Numerous distorted spheres and dumbbells with large central and/or small inclusions. Some spheres without inclusions have the appearance of recrystallized structures	Inclusions unchanged; some well defined blow holes	No inclusions; openings but no well defined blow holes
12023, 8 fines (b)	As above	Same as above	No inclusions; well defined blow holes; one thick irregular glass fragment containing a bead 21 μ m in diameter, with inclusion
12033, 8 fines (c)	As above	No inclusions; no well defined blow holes	No blow holes; beads almost unrecognizable
12042, 13 fines (d)	As above, with the exception of few large central inclusions, numerous small inclusions	Inclusions unchanged, many small blow holes	No inclusions; beads deformed; irregular and well defined holes are visible
12025, 60 fines. Core, 9 cm below surface (e)	Spheres covered with fine particulate matter; broken and deformed beads; inclusions and well defined blow holes	Small blow holes, inclusions	No inclusions; no blow holes; beads deformed
12028, 207 fines. Core, 39 cm below surface (f)	As above; one well formed 77 μ m elongated dumbbell with inclusion	Same as above	Same as above
Rock 12022, 81 (g)	No inclusions; no blow holes	No inclusions; no blow holes	Not examined

(a) Documented fines collected near Lunar Module. (b) Documented fines from Lunar Environment Sample Container; collected from the bottom of 15 cm trench inside north rim of Sharp Crater. (c) This sample is regarded to be an ash layer; documented and dug near north rim of Head Crater. (d) Documented collection in an area of "wrinkled texture" near rim of Surveyor Crater. (e) Top of double core tube No. 2010, collected on rim of 10 m diameter crater south of Halo Crater. (f) Bottom of double core tube No. 2012 (same collection site). (g) Inside chip from documented olivine dolerite. This rock was partially buried and was located approximately 120 m north-west of the Lunar Module.

Sample locations (a)–(g) are taken from ref. 10.

were artefacts resulting from the He pyrolysis technique. In a control experiment benzene was pyrolysed in He at 600° C and yielded traces of, predominantly, biphenyl with lesser amounts of naphthalene. Pre-Cambrian rock samples which had given He pyrolysis products similar to those found in the lunar samples were subjected to vacuum pyrolysis at 500° C and 600° C using a liquid nitrogen cold trap and showed only ions of $m/e = 76, 78, 91, 92, 154, 177$ which indicate the presence of benzene, toluene, alkyl benzenes, biphenyl, and so on, plus smaller molecular weight hydrocarbons below $m/e = 78$. It was also determined in a separate experiment with this cold trap vacuum pyrolysis technique that biphenyl can be destroyed by pyrolysis at temperatures of 700° C and above. The results showed degradation and intramolecular rearrangement to products such as naphthalene.

Trace amounts of fluorocarbon compounds were also observed in some of the pyrolysed samples, but these were probably 'Teflon' contaminations. A 'Teflon' control pyrolysis, using 'Teflon' supplied and used by the NASA Manned Spacecraft Center, showed these same fluorocarbon compounds. An Ottawa quartz sand control, which was exposed in the same cabinet at the LRL where the lunar samples were processed, was also He pyrolysed; Fig. 2E shows its gas chromatogram. Note that it shows no compounds beyond the small benzene peak. It has been reported (R. B. Erb, personal communication) that the container in which this sample was shipped was cleaned with benzene.

Amino-acid Analyses

Three samples of lunar fines, 12001, 34 (4.1703 g), 12023, 8 (9.0000 g) and 12033, 8 (9.0015 g), were analysed for their amino-acid content by hot water Soxhlet extraction and by ion exchange column chromatographic techniques⁷. Complete parallel procedure blanks were run at the same time. The water used for the extraction was triple distilled and all glassware was cleaned with acid. Before the ion exchange chromatography the extracts and the corresponding blanks were checked for bacterial contamination. The cultures showed no growth.

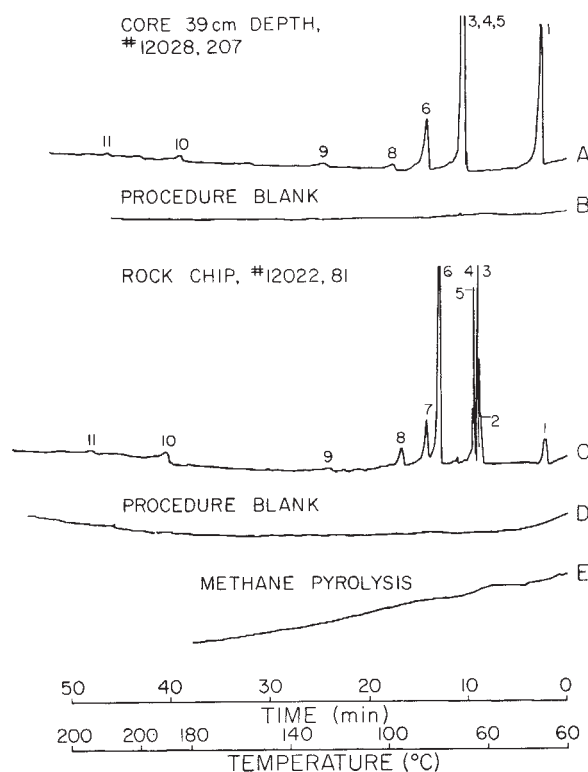


Fig. 3 Gas chromatograms of the pyrolysis products of (A) lunar core, 39 cm depth (sample 12028, 207); (C) lunar rock chip (sample 12022, 81), and (E) CH_4 pyrolysis over pre-pyrolysed lunar fines (sample 12001, 34). Peak identities, all traces: 1, H_2 , CH_4 , CO; 2, CH_4 ; 3, C_2H_4 ; 4, C_3H_6 ; 5, C_4H_8 ; 6, benzene; 7, thiophene; 8, toluene; 9, styrene; 10, naphthalene; 11, biphenyl. Additionally, on trace A: 3 also contains C_2F_4 , $\text{C}_2\text{H}_2\text{F}_2$; 4, $\text{C}_4\text{F}_4\text{H}_2$; 5, C_3F_6 ; 6, C_4H_6 ; and on trace C: 1, CO absent; 3, CH_4 , CO, CO_2 ; 4, CO_2 , Ne, possibly COS; 5, CO_2 , C_3H_6 . Compounds 7 and 9–11 can be artefacts.

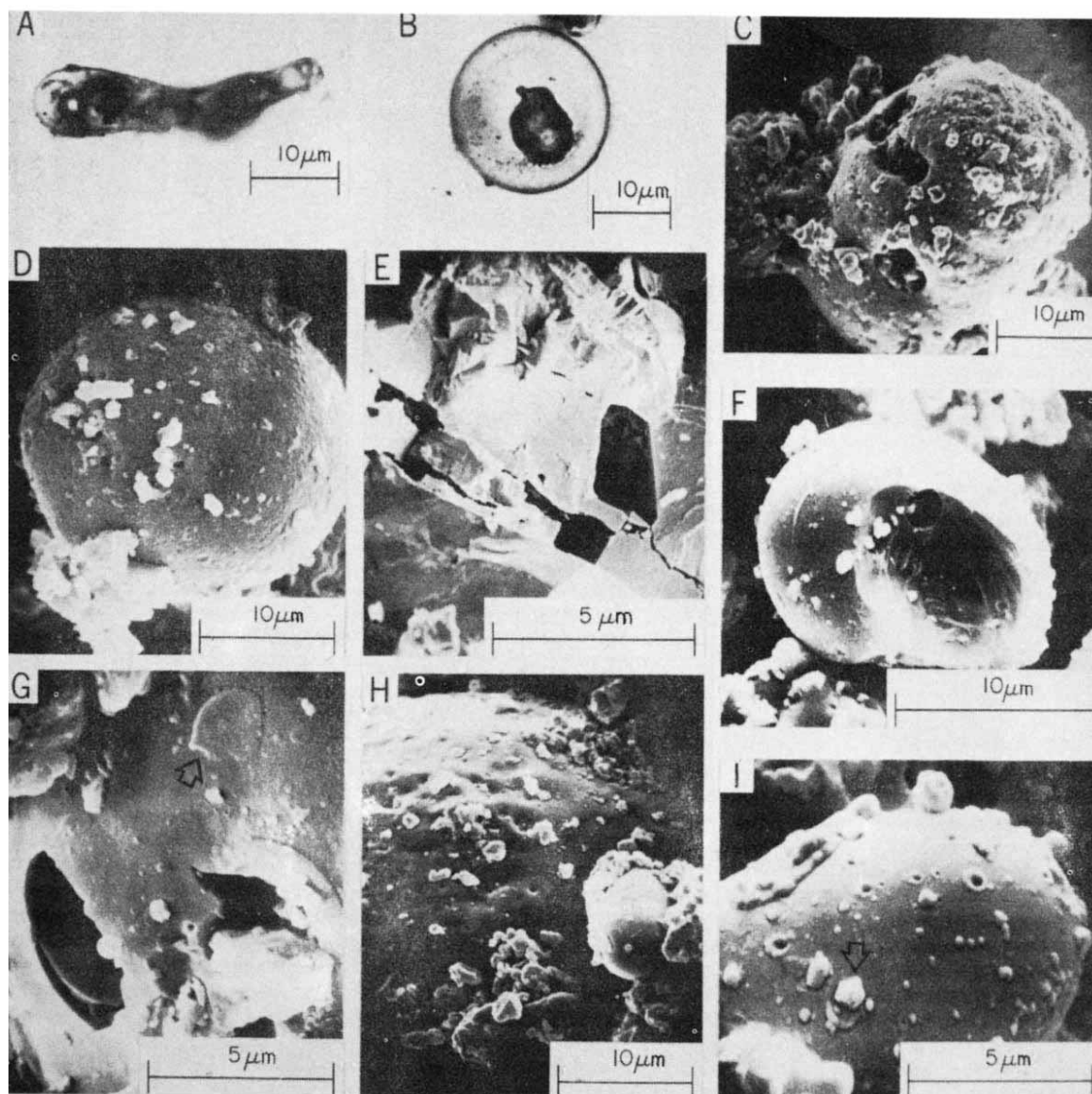


Fig. 4 *A* and *B*, Transmitted light photomicrographs of "twisted" glass dumbbell and glass sphere with opening, respectively; *C* and *D*, scanning electron micrographs of beads after pyrolysis at 1,084° C; *E*, freshly broken surface of interior rock chip (note absence of vesicles); *F*, partially broken glass bead showing cavity near the surface; *G*, a portion of a thin walled glass bead with broken openings (note arrow pointing to a flat object, probably produced by collision with a molten particle); *H*, part of a glass bead showing what appear to be several blow holes; *I*, one end of a glass dumbbell showing holes produced by hypervelocity impacts. Note arrow pointing to larger and slower velocity particle which caused imprint but did not penetrate the surface.

The analyses of the three lunar samples indicated the presence of only traces of amino-acids in the low p.p.b. range. After subtracting the amino-acids in the blanks from the lunar sample chromatograms, samples 12001, 23 and 12023, 8 showed traces of urea, aspartic acid, threonine, serine, glycine, alanine and ornithine. The highest concentration of amino-acids occurred in sample 12001, 34. NASA reported that this sample was likely to be contaminated. The amino-acid distribution found is similar to that expected from hand contamination⁸. In sample 12023, 8 the total amount of amino-acids is approximately 1/20 of that in sample 12001, 34 and the distribution again suggests hand contamination. In sample 12033, 8 the amino-acid content appeared to be zero. Urea, however, was present even in this sample. It seems, therefore, that these samples did not contain the amino-acids found in Apollo 11 fines³.

Electron Microscopic Studies

All samples of lunar fines were studied before and after pyrolysis at 700° C in He and at 1,084° C in vacuum, using a

Leitz Ortholux microscope with a transmitted light and oil immersion objective having a magnification of 950.

In the fines, silicate spheres, "tear drop", and dumbbell shaped particles (with and without large inclusions) were commonly found among the irregularly shaped mineral matter. The spheres and dumbbells resembled the spheres and dumbbells found in the Apollo 11 fines but there were some subtle differences. In all of the untreated fines which we examined, many of the spheres and dumbbells appeared to be more irregular than the almost perfect spheres found in the Apollo 11 fines. Some particles had relatively large inclusions, while other spheres had no inclusions at all; others had an appearance similar to the heat treated (1,200° C) Apollo 11 spheres. Only the untreated sample 12042, 13 contained very few large inclusions, although numerous smaller inclusions were also observed in this sample. In the same sample, an elongated dumbbell (Fig. 4*A*) and a bead enclosed in an irregularly shaped glass fragment were found. In the untreated sample 12033, 8, reported to be an ash layer⁹, there was an elongated and twisted dumbbell, which gave the appearance of having

started to elongate and melt on heating. There was also a small hole at one end of this particle.

Morphologically, the core samples were no different from the surface fines, with the possible exception that many of the glass beads were covered with attached, fine, particulate matter. The glass beads from the core samples also showed numerous well defined cavities and inclusions. The lunar rock showed no vesicles or holes. The morphological effects of pyrolysis on all the samples at 700° C and 1,084° C are summarized in Table 2.

Lunar samples 12023, 8, 12042, 13, the core sample from a depth of 39 cm, and a freshly broken surface of the rock chip, were examined by a scanning electron microscope. The core sample and sample 12023, 8 were also examined after pyrolysis at 1,084° C. The fines were mounted on the electron microscope pedestal with a drop of absolute methanol. The rock chip was mounted with double adhesive tape. All sample preparations for electron microscopy were performed in the clean room of the Naval Weapons Center at China Lake, California. The pyrolysed fines showed much less tendency to adhere to the bare pedestal surface than did the untreated samples. The samples were coated with an ~500 Å thick layer of gold-palladium alloy by vacuum deposition³ and then inserted in the scanning electron microscope.

The pyrolysis at 1,084° C seems to have led to the development of rough surfaces on the beads (Fig. 4C and D). Smooth beads were difficult to find and larger holes were more noticeable in the beads with rough surfaces than in the unpyrolysed beads. These effects might indicate softening of the glass accompanied by degassing at the pyrolysis temperature.

The freshly broken surface of the lunar rock showed no vesicles, only massive crystalline structure with fissures which were possibly arranged along cleavage planes (Fig. 4E); conchoidal fractures were also present. Other untreated samples showed a number of interesting features. From the particle illustrated in Fig. 4F, a chip was removed, probably by impact, resulting in a conchoidal surface and revealing an internal cavity very near to the original surface of this glass bead. Fig. 4G shows part of a thin walled glass bead with irregular shaped broken holes and a plaque (note arrow), probably resulting from a completely molten, impacting object. Fig. 4H is particularly noteworthy because it contains numerous small openings which resemble blow holes. The end of a

dumbbell shown in Fig. 4I is covered with what appear to be impact craters with lips, which might have been caused by impacts from hypervelocity particles of about 0.1–0.2 µm in size. Lower velocity and larger impacting particles, ~1.0 µm in diameter, show impact prints but remained attached to this dumbbell without penetrating its surface (note arrow).

There seem to be subtle differences between the morphologies of the Apollo 11 and 12 lunar fines. The abundance of what appear to be degassing blow holes, distorted particles and so on may suggest a different thermal history at the Apollo 12 landing site. It appears that the carbon compounds are present in some of the enclosures of the fines, and there seem to be carbon compounds dissolved in the lunar rock (interior) that we examined. This lunar rock contained no vesicles. One can account for the carbon in the Apollo 12 fines partially from solar wind emplacement¹¹. It is, however, difficult to account for the carbon in the interior of the rock by this mechanism. Degassing of the Moon could be one mechanism which might account for the carbon in the interior of igneous lunar rocks.

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¹ Silver, L. T., *Geol. Soc. Amer. Abstract*, **2**, 684 (1970).

² Wasserburg, G. J., and Papanastassiou, D. A., *Geol. Soc. Amer. Abstract*, **2**, 715 (1970).

³ Nagy, B., Drew, C. M., Hamilton, P. B., Modzeleski, V. E., Murphy, Sr. M. E., Scott, W. M., Urey, H. C., and Young, M., *Science*, **167**, 770 (1970).

⁴ Nagy, B., Scott, W. M., Modzeleski, V. E., Nagy, L. A., Drew, C. M., McEwan, W. S., Thomas, J. E., Hamilton, P. B., and Urey, H. C., *Nature*, **225**, 1028 (1970).

⁵ Scott, W. M., Modzeleski, V. E., and Nagy, B., *Nature*, **225**, 1129 (1970).

⁶ Oro, J., and Han, J., *J. Gas Chromatog.*, **5**, 480 (1967).

⁷ Hamilton, P. B., *Anal. Chem.*, **35**, 2055 (1963).

⁸ Hamilton, P. B., *Nature*, **205**, 284 (1965).

⁹ *NASA Lunar Sample Information Catalog Apollo 12*, 121 (1970).

¹⁰ *NASA Apollo 12 Preliminary Science Report*, sample description on pp. 137–144, map on p. 33 (1970).

¹¹ Moore, C. B., Larimer, J. W., Lewis, C. F., Delles, F. M., and Gooley, R. C., *Geol. Soc. Amer. Abstract*, **2**, 628 (1970).

¹² Nagy, B., *Geotimes*, **15**, 18 (1970).

¹³ Preti, G., Murphy, R. C., and Biemann, K., *Apollo 12 Lunar Science Conference*, Houston, Texas (1970).

Mesozoic Palaeomagnetic Stratigraphy

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Mesozoic polarity measurements suggest that there are two long periods of predominantly normal polarity from Upper Triassic to Upper Jurassic and during the Cretaceous. The former provides a simple explanation of the North Atlantic magnetic quiet zones. Magnetic stratigraphic nomenclature is proposed to cover polarity stratigraphy on the geological time scale.

PALAEOMAGNETIC measurements and K–Ar dating of volcanic rocks have established a detailed geomagnetic polarity time scale for the past 4.5 m.y. (ref. 1). Similar reversal sequences are found in deep sea sediment cores², the longest of which are estimated to provide continuous polarity information back to about 10 m.y. (ref. 3). These polarity sequences show two essential features. There are periods of constant polarity of the order of 10⁶ yr called magnetic epochs⁴, within which are much shorter periods during which the polarity changed for only 10⁵ yr or less. These shorter periods are called magnetic events⁵. Magnetic epochs have been named after various geomagnetists⁵ and there are four established for the past 4.5 m.y., called the Brunhes, Matuyama, Gauss and Gilbert Epochs. Magnetic events are named after the places where they were first identified⁵.

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The study of marine magnetic anomalies and their interpretation in terms of the seafloor spreading hypothesis⁶ and reversals of the Earth's magnetic field⁷ has made it possible to extrapolate the land-based polarity time scale back to about 70 m.y. (ref. 8). According to this time scale the Cenozoic, a period of 65 m.y., has 160 polarity intervals and the geomag-

netic field has, on average, spent equal amounts of time in the normal and reversed states. On the other hand, during the Late Carboniferous and Permian, there was a period of about 50 m.y. during which the field remained almost continuously in the reversed state. Irving and Parry⁹ named this the Kiaman Magnetic Interval, because it was in a sample from the Upper

Marine Latites collected near Kiama, New South Wales, that Mercanton¹⁰ first observed reversely magnetized rocks of this age.

An analysis of the polarities observed in nearly 1,100 world-wide palaeomagnetic investigations of Phanerozoic rocks¹¹ has revealed an interesting pattern in the behaviour of the polarity of the geomagnetic field, which appears to have cycled through a long period of about 350 m.y. During the Upper Palaeozoic the field was predominantly reversed, while during the Mesozoic it was predominantly normal. Apart from the Lower Triassic where frequent reversals have already been observed¹²⁻¹⁴, the Mesozoic geomagnetic field was in the normal state about 75% of the time. Helsley and Steiner¹⁵ have already proposed a polarity time scale for the Cretaceous and have provided evidence for an interval of normal polarity of length similar to that of the Kiaman Magnetic Interval. Opdyke and McElhinny¹⁶ first suggested that the Upper Triassic and much of the Jurassic was predominantly normal, but that an important reversal existed at the Triassic-Jurassic boundary and that this reversal could be an important horizon for geological correlation. Brock¹⁷ summarized all the data from palaeomagnetic studies in southern Africa relating to this proposal and was able to indicate the sequence of polarity change in three instances. Burek¹⁸ then proposed a preliminary Permo-Triassic polarity time scale, also suggesting a predominantly normal interval of Late Triassic to Early Jurassic age.

In Fig. 1 all worldwide palaeomagnetic polarity information for the Triassic and Jurassic is summarized. The information is compiled from the tables of Irving¹⁹ and McElhinny²⁰⁻²³ and the synoptic tables of USSR palaeomagnetic data²⁴. Only those investigations where the rocks studied have been assigned to one of the major subdivisions of each geological period (Lower, Middle or Upper) have been used. Where only radiometric information is available, the rocks are assigned to their stratigraphic position according to the time scale of the Geological Society of London²⁵. For this purpose it has been necessary to compare ages determined by both Rb-Sr and K-Ar methods. Following the general practice, all Rb-Sr ages have been converted to the decay constant $\lambda = 1.475 \times 10^{-11} \text{ yr}^{-1}$ for this comparison. We have placed the Triassic-Jurassic boundary at 190 m.y. The results are presented in three columns for Europe-Siberia, North America and Gondwanaland. Bracketed numbers greater than 100 after any formation name refer to a radiometric age. References to the various tables of data are as follows. Numbers with a decimal point refer to the corresponding numbers in Irving's tables¹⁹. McElhinny's tables (VIII, IX, X, XI)²⁰⁻²³ are referred to by the table number, an oblique line and then the number of the table entry (for example, 9/76 means table 9, entry 76). Entries in the Russian tables²⁴ are similarly indicated by R followed by an oblique line and the number of the entry.

Our overall interpretation of these data is given in Fig. 2, where we have also added the Cretaceous polarity time scale suggested by Helsley and Steiner¹⁵. The results strongly suggest the existence of a predominantly normal period extending from the Upper Triassic (about 200 m.y. ago) to the Upper Jurassic (about 150 m.y.). The existence of these long periods of predominantly one polarity and length about 5×10^7 yr confirms the suggestion of McMahon and Strangway²⁶ that the time scales of polarity intervals of the geomagnetic field are 5×10^7 yr, 10^6 yr and 10^5 yr or less. On the long time scale, it seems unlikely that it will be possible to distinguish in the first instance between magnetic epochs and events as has been done for the past 4.5 m.y. (ref. 1). We therefore propose a new magnetic stratigraphy to cover the geological time scale.

It should be possible to divide geological time into a number of "magnetic intervals", which last 10^7 to 10^8 yr. These intervals might be long periods of time during which either very occasional or no polarity changes occurred (such as the Kiaman Magnetic Interval) or they might be long periods of time during which polarity changes occurred quite regularly. A number of such intervals are defined in Fig. 1 for the Mesozoic. The

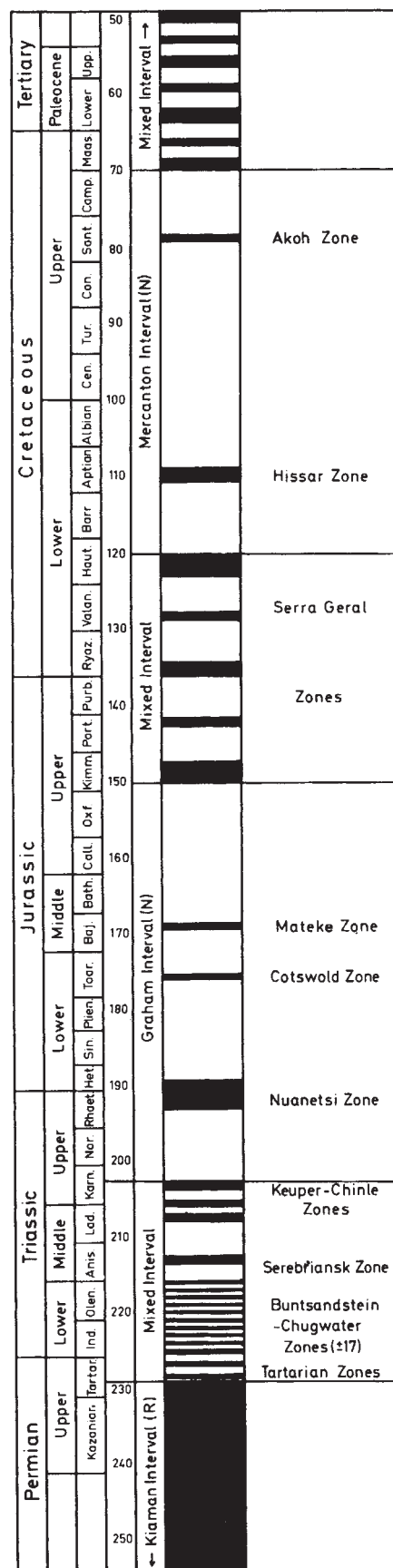


Fig. 2 Proposed geomagnetic polarity time scale for the Mesozoic. Shaded regions are reversed polarity, unshaded regions are normal polarity.

well known Late Palaeozoic interval was named after the place where reversely magnetized rocks of this age were first discovered. It might not, however, necessarily be appropriate to use this criterion in naming the other magnetic intervals, for it will often be difficult to establish precedence. We believe it might be just as appropriate to name some of these intervals after people, as in the case with magnetic epochs over the past few million years. We have therefore named the predominantly normal interval ranging from Upper Triassic to Upper Jurassic the Graham Magnetic Interval in recognition of the pioneering work in palaeomagnetism by John W. Graham over the past 25 yr. This is especially appropriate since it appears he was the first person to undertake palaeomagnetic investigations of Upper Triassic rocks anywhere in the world, when he studied rocks of this age in the United States²⁷. The long normal interval during the Cretaceous extends from around 120 m.y. up to 70 m.y. and has been named the Mercanton Magnetic Interval after P. L. Mercanton who first made palaeomagnetic polarity measurements on rocks of many different ages from different parts of the world and established that reversals occurred irrespective of locality and age^{10,28-30}. We have not yet attempted to name any of the intervals of predominantly mixed polarity, the oldest of which lies between the Kiaman and Graham Magnetic Intervals, covering the Lower and Middle Triassic and lasting about 25 m.y. Between the Graham and Mercanton Intervals a mixed interval about 30 m.y. in length is suggested. The longest of the mixed intervals corresponds to the past 70 m.y.

The shorter periods of length 10^6 yr or less, when the field temporarily changed polarity, have been called magnetic zones. The term has been chosen because in practice it will not be possible to distinguish between epochs and events; the term zone is intended to cover both of these possibilities. These zones are named after the place or rock formation of their discovery or association. Previously Burek¹⁸ attempted to define a Triassic palaeomagnetic stratigraphy in which various magnetic events were distinguished. We do not now feel that the terminology used then is the best approach and our proposed stratigraphy and nomenclature is intended to update, supersede and extend that of Burek¹⁸. A number of magnetic zones have been named after the various rock formations. The Nuanetsi Zone at the Triassic-Jurassic boundary follows the original suggestion of such a reversed zone at this horizon by Opdyke and McElhinny¹⁶, and which has been elaborated by Brock¹⁷. It is found in a 700 m lava sequence in the Nuanetsi lavas of Rhodesia dated at 194 ± 12 m.y., and its beginning is located in the associated Marangudzi Ring Complex of age 190 ± 5 m.y. where the polarity change from normal to reversed is recorded¹⁷. The zone is related to the reverse magnetization found in the Upper Triassic-Lower Jurassic Pachmarhi formation of India³¹. Girdler³² found a reversed zone in the Upper Lias Cotswold Sands of England, a horizon that appears to be related to the reversed polarity found in the White Mountain Volcanics dated at 180 m.y. (ref. 33). Recent results from two boreholes in the Jurassic coal bed of the Kuznetsk Basin³⁴ also show that the Lower Jurassic is predominantly normal and that a reversed horizon exists only in the Toarcian. This observation is confirmed by measurements on Jurassic rocks from the coast of Anabar Bay, northern Siberia³⁵. An accurately dated reversed zone in the Middle Jurassic is found in the Mateke Hills Complexes of Rhodesia, dated at 168 ± 4 m.y., in which the change from reversed to normal polarity is also recorded¹⁷. The two reversed zones in the Mercanton Interval follow Helsley and Steiner¹⁵. They are here named after the South-West Hissar Range³⁶ of the USSR and the Akoh Formation³⁷ of Japan respectively. More recent information from igneous rocks in Hungary³⁸ slightly modifies the Cretaceous polarity time scale of Helsley and Steiner¹⁵. The reversed zones at the base of the Cretaceous are therefore continued up into the Hauterivian in Fig. 2.

Previously it has been suggested that a narrow band of normal polarity occurs within the Kiaman Magnetic Interval³⁹

near the Permo-Carboniferous boundary. This was named the Oak Creek Event by McElhinny³⁹, but was later also named the Graham Event by Burek¹⁸. On the terminology suggested here this band should now be referred to as the Oak Creek Zone of the Kiaman Magnetic Interval, since this name has precedence. Khramov and his co-workers in the USSR^{40,41} have attempted to produce a palaeomagnetic stratigraphy for the whole of the Palaeozoic, based largely on extensive studies of Palaeozoic sections in Siberia. Their work suggests that another predominantly reversed interval existed before the Kiaman Interval extending over roughly 50 m.y. from Middle Devonian to Middle Viséan. This interval contains only one small normal zone at the Famennian-Frasnian boundary.

A characteristic feature of the ocean magnetic anomalies in the North Atlantic are the so called magnetic quiet zones whose boundaries lie 2,000 to 2,500 km from the axis of the mid-Atlantic Ridge⁴². A number of explanations for these magnetic smooth zones have been put forward, including lack of magnetic material in the crust⁴³, stages of continental rifting producing plateau basalts with smaller specific intensities than submarine dykes⁴⁴, magnetization at low geomagnetic latitudes⁴⁵, and spreading during periods of few or no geomagnetic reversals^{18,42,46}. Heirtzler and Hayes⁴² suggested the quiet zones reflected the Kiaman Magnetic Interval. Burek¹⁸ suggested a Late Triassic to Early Jurassic age for initial rifting of the Atlantic Ocean, correlating the quiet zones with the lower part of what we have now termed the Graham Magnetic Interval. More detailed magnetic anomaly profiles^{46,47} over the quiet zones correlate well with this interval. Emery *et al.*⁴⁶ in attempting to correlate the quiet zones with the Kiaman Magnetic Interval required that the spreading rate decrease from 1.43 cm yr^{-1} to 0.80 cm yr^{-1} between Joides site 9 and the continental shelf. A constant spreading rate, however, places the continental slope at about 200 m.y. and the quiet zone lies between 160 and 200 m.y., within the Graham Magnetic Interval. The rather large anomaly at the continental slope corresponds to the Nuanetsi Zone and the two events within the quiet zone may be correlated with the Cotswold and Mateke Zones. There seems no reason therefore to invoke more elaborate explanations⁴³⁻⁴⁵ for the quiet zones when a relatively simple one exists.

If the slope anomaly denotes the line along which North America rifted from North-West Africa and Europe, then our interpretation of the magnetic smooth zones dates this rifting at about 200 m.y. ago. This interpretation is supported by tectonic events of Rhaetic age⁴⁸ in North America (Palisades orogeny and associated volcanism of the Newark Graben system) and by evaporite formation of Rhaetic age in three coastal basins of North-West Africa⁴⁹, which are correlated with diapirs originating in the basal sediments in the Canary Islands⁵⁰.

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¹ Cox, A., *Science*, **163**, 327 (1969).

² Ninkovitch, D., Opdyke, N., Heezen, B. C., and Foster, J. H., *Earth Planet. Sci. Lett.*, **1**, 476 (1966).

³ Foster, J. H., and Opdyke, N. D., *J. Geophys. Res.*, **75**, 4465 (1970).

⁴ Cox, A., Doell, R. R., and Dalrymple, G. B., *Nature*, **198**, 1049 (1963).

⁵ Cox, A., Doell, R. R., and Dalrymple, G. B., *Science*, **144**, 1537 (1964).

⁶ Hess, H. H., in *Petrologic Studies*, 599 (Geol. Soc. Amer., 1962).

⁷ Vine, F. J., and Matthews, D. H., *Nature*, **199**, 947 (1963).

⁸ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).

⁹ Irving, E., and Parry, L. G., *Geophys. J. Roy. Astron. Soc.*, **7**, 395 (1963).

¹⁰ Mercanton, P. L., *Terr. Magn. Atmos. Elect.*, **31**, 187 (1926).

¹¹ McElhinny, M. W., *Science*, **172**, 157 (1971).

¹² Picard, M. D., *Amer. Assoc. Petr. Geol. Bull.*, **48**, 269 (1964).

¹³ Burek, P. J., *Geol. Jahrb.*, **84**, 591 (1964).

¹⁴ Helsley, C. E., *Geol. Soc. Amer. Bull.*, **80**, 2431 (1969).

¹⁵ Helsley, C. E., and Steiner, M. B., *Earth Planet. Sci. Lett.*, **7**, 325 (1969).

¹⁶ Opdyke, N. D., and McElhinny, M. W., *Trans. Amer. Geophys. Union*, **46**, 65 (1965).

- ¹⁷ Brock, A., *J. Geophys. Res.*, **73**, 1389 (1968).
- ¹⁸ Burek, P. J., *Amer. Assoc. Petr. Geol. Bull.*, **54**, 1120 (1970).
- ¹⁹ Irving, E., in *Palaeomagnetism* (Wiley, New York, 1964).
- ²⁰ McElhinny, M. W., *Geophys. J. Roy. Astron. Soc.*, **15**, 409 (1968).
- ²¹ McElhinny, M. W., *Geophys. J. Roy. Astron. Soc.*, **16**, 207 (1968).
- ²² McElhinny, M. W., *Geophys. J. Roy. Astron. Soc.*, **18**, 305 (1969).
- ²³ McElhinny, M. W., *Geophys. J. Roy. Astron. Soc.*, **20**, 417 (1970).
- ²⁴ Khramov, A. N., and Ye Sholpo, L., in *Paleomagnetism* (Nedra Press, Leningrad, 1967).
- ²⁵ Harland, W. B., Smith, A. G., and Wilcock, B., *Quart. J. Geol. Soc. London*, **120s** (1964).
- ²⁶ McMahon, B. E., and Strangway, D. W., *Science*, **155**, 1012 (1967).
- ²⁷ Graham, J. W., *J. Geophys. Res.*, **60**, 329 (1955).
- ²⁸ Mercanton, P. L., *CR Acad. Sci. Paris*, **180**, 859 (1926).
- ²⁹ Mercanton, P. L., *CR Acad. Sci. Paris*, **192**, 978 (1931).
- ³⁰ Mercanton, P. L., *CR Acad. Sci. Paris*, **194**, 1371 (1932).
- ³¹ Wensink, H., *Palaeogeog. Palaeoclim. Palaeoecol.*, **5**, 323 (1968).
- ³² Girdler, R. W., *Nature*, **184**, 550 (1959).
- ³³ Opdyke, N. D., and Wensink, H., *J. Geophys. Res.*, **71**, 3045 (1966).
- ³⁴ Aparin, V. P., Ya Vlassov, A., and Kirillov, V. M., *Geomag. and Aeronomy*, **9**, 634 (1969).
- ³⁵ Pospelova, G. A., Ya Larionova, G., and Anuchin, A. V., *Intern. Geol. Rev.*, **10**, 1108 (1968).
- ³⁶ Abdullayev, Kh. A., *Akad. Nauk SSSR Izv. Geophys. Ser.*, **919** (1964).
- ³⁷ Sasajima, S., and Shimada, M., *Geol. Soc. Japan J.*, **72**, 503 (1966).
- ³⁸ Dagley, P., and Ade-Hall, J. M., *Geophys. J. Roy. Astron. Soc.*, **20**, 65 (1970).
- ³⁹ McElhinny, M. W., *Spec. Publ. Geol. Soc. Australia*, **2**, 61 (1969).
- ⁴⁰ Khramov, A. N., Rodionov, V. P., and Komissarova, R. A., in *The Present and Past of the Geomagnetic Field*, 206 (Nauka Press, Moscow, 1965).
- ⁴¹ Khramov, A. N., *Akad. Nauk SSSR Izv. Earth Phys. Ser.*, **86** (1967).
- ⁴² Heirtzler, J. R., and Hayes, D. E., *Science*, **157**, 185 (1967).
- ⁴³ Drake, C. L., and Nafe, J. E., *Geophys. Monog. Amer. Geophys. Union*, **12**, 174 (1969).
- ⁴⁴ Irving, E., *Canad. J. Earth Sci.*, **7**, 1528 (1970).
- ⁴⁵ Vogt, P. R., Anderson, C. N., Bracey, D. R., and Schneider, E. D., *J. Geophys. Res.*, **75**, 3955 (1970).
- ⁴⁶ Emery, K. O., Uchupi, E., Phillips, J. D., Bowin, C. O., Bunce, E. T., and Knott, S. T., *Amer. Assoc. Petr. Geol. Bull.*, **54**, 44 (1970).
- ⁴⁷ Rona, P. A., Brakland, J., and Heirtzler, J. R., *J. Geophys. Res.*, **75**, 7412 (1970).
- ⁴⁸ Burek, P. J., *Geol. Soc. Amer. Meet. Prog.*, **7**, 24 (1969).
- ⁴⁹ Reyre, D., in *Atlantic Coast Symp., Pt. I*, 304 (Assoc. African Geol. Surv., 1964).
- ⁵⁰ Rona, P. A., *Nature*, **224**, 141 (1969).

Disruption by Carcinogens of the Hormone Dependent Association of Membranes with Polysomes

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It can be shown by enzyme assay that the effect of a variety of carcinogens is the degranulation of the endoplasmic reticulum.

THE biochemical basis of the carcinogenic activity of chemical substances has not been established in spite of the enormous amount of experimental effort expended¹⁻³. It is now clear that most chemical carcinogens need to be transformed either to substances possessing electrophilic reactivity or to radical intermediates before the train of events leading to tumour formation is initiated⁴⁻⁷. The microsomal "hydroxylase" system, central to this activation for a number of compounds, catalyses reactions involving N-oxygenation of aromatic amines⁸⁻¹¹, dealkylation of substituted amines and nitrosamines¹²⁻¹⁴ and probably ring and side chain oxygenation of polycyclic aromatic hydrocarbons^{13,15-17}. The hepatotoxicity of carbon tetrachloride is also believed to depend on activation, probably to a radical intermediate¹⁸. The radical or electrophilic products are known to interact with macromolecules such as nucleic acids and proteins¹⁹⁻²¹. The microsomal "hydroxylase" complex is tightly associated with microsomal membranes deriving from the endoplasmic reticulum of the intact cell^{13,22}.

If the target of the reactive metabolites of carcinogens is on the membrane itself, the species produced during activation would not need to survive the rather unfavourable environment of the cytosol containing as it does powerful nucleophiles such as amino and sulphhydryl groups on both small molecules and macromolecules.

There is some controversy as to whether aflatoxin B₁ requires activation to a proximate carcinogen^{4,23,24}. In our opinion the balance of evidence favours the view that prior activation is not a requirement for the carcinogenicity of this compound and indeed it seems probable that interaction with the microsomal enzymes leads to its detoxification. It has been demonstrated that aflatoxin B₁ interacts with the membranes of rat liver endoplasmic reticulum to interfere with membrane-polysome interactions both *in vivo* (B. R. R., K. R. Rees, and D. J. W., unpublished) and *in vitro*²⁵. Evidence has been presented that steroid hormones are involved in the operation of membrane-ribosome binding sites²⁶. Low concentrations of oestradiol promote the binding of male polysomes to smooth surface male membranes and similarly testosterone serves the same function in female tissue²⁹. In these experiments oestradiol can be replaced by an ethyl acetate extract of female polysomes and testosterone by a similar extract of male polysomes⁴⁰. Interaction of aflatoxin B₁ with membranes prevents their association with polysomes promoted by steroid hormones and the carcinogen will also effect the degranulation of rough

membranes^{25,26}. Both rough and smooth surfaced membranes can be protected from the action of aflatoxin B₁ *in vitro* by oestradiol in the male and testosterone in the female. Direct physicochemical measurements show that aflatoxin B₁ occupies or destroys a site on the membrane specific for oestradiol in the male and testosterone in the female³⁸.

It is of great significance that in an apparently separate system and with two other carcinogens (dimethylaminoazobenzene and 3-methyl cholanthrene) Litwack and his co-workers have demonstrated *in vivo* association of the carcinogen with a steroid binding protein specific for cortisol metabolites in male liver²⁷. On the basis of the observations reported above it is reasonable to make the tentative suggestion that chemical carcinogenesis is analogous to hormone controlled development and differentiation. If this hypothesis has any validity, the appropriate hormone should protect the initial primary target against the action of the carcinogens. We describe a system involving steroid hormones which interacts with every carcinogen tested and which can be protected by the specific hormone involved.

Membrane Degranulation

Membrane degranulation *in vitro* may be conveniently monitored by measuring the apparent activity of a membrane bound disulphide interchange enzyme, normally masked by bound ribosomes^{25,26,28}. This technique was used to test the ability of some carcinogens and their non-carcinogenic analogues to effect degranulation of microsomal membranes from rat liver. Table 1 summarizes some of the results of these experiments using a system fortified with NADPH and post-microsomal supernatant. Fig. 1 illustrates the results of one

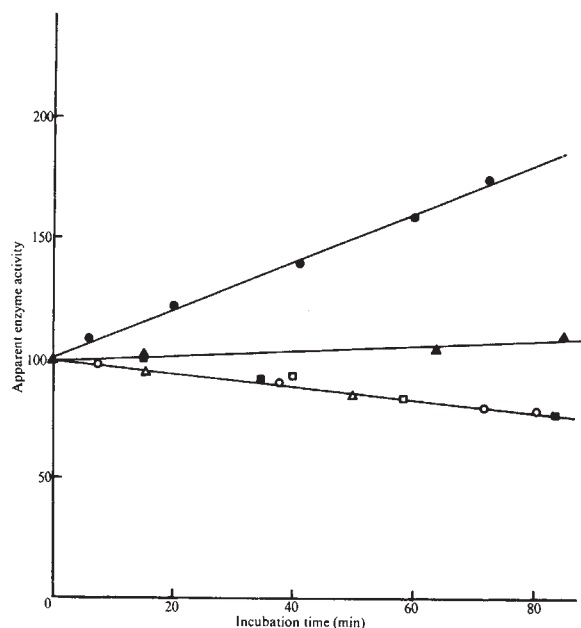


Fig. 1 The increase in the activity of the disulphide interchange enzyme caused by incubating microsomal membranes with 2-aminochrysene in the presence of NADPH. Microsomal membranes (about 8 mg protein ml.⁻¹) were incubated with 2-aminochrysene (5 µg ml.⁻¹) in the presence of 1 mM NADPH, 50 mM Tris, 25 mM KCl, 5 mM MgCl₂, and 0.25 M sucrose, at pH 7.3. Post microsomal supernatant was added to 10% of the final volume of incubate and the incubation carried out at 21° C. The activity of the membranes was monitored in the standard system (●), in the presence of naphthalene, 10 µg ml.⁻¹ (▲), SKF 525A, 1 mM (△), and oestradiol, 1 µg ml.⁻¹ (□). A similar incubation mixture was set up containing no NADPH (■) and one containing 6-aminochrysene in place of the 2-aminochrysene at the same concentration (○). The enzyme activity is expressed as a percentage of the initial value.

Table 1 Rate of Increase of Apparent Disulphide Interchange Enzyme Activity of Microsomal Membranes from Rat Liver on Incubation with the Substances indicated in the Presence of NADPH

Substance tested *	Rate of enzyme activation †	Carcinogenicity (refs. 11, 29, 31, 33)
Naphthalene	0	Nil
2-Naphthylamine	0-3	±
Anthracene	0	Nil
2,7-Diaminofluorene	8	+
1,2-Benzanthracene	5	+
1,2-Benzanthraquinone	8	+
2-Aminochrysene	8	+
6-Aminochrysene	0	Nil
3-Aminopyrene	9	+
3,4-Benzpyrene	20	+
Benzo[b]chrysene	0	Nil
3,4,8,9-Dibenzpyrene	13	+
Carbon tetrachloride (15 µg ml. ⁻¹)	8	+
Aflatoxin B ₁ ‡ (10 µg ml. ⁻¹)	10	+

* Incubated at 20° C with microsomal membranes (10 mg protein ml.⁻¹) at a concentration of 5 µg ml.⁻¹ (unless otherwise stated) in the presence of 1 mM NADPH, 50 mM Tris, 25 mM KCl, 5 mM MgCl₂ and 250 mM sucrose, at pH 7.3. Post microsomal supernatant was added to 10% final volume.

† Percentage of total enzyme activity released/h.

‡ Absence of NADPH.

experiment, in which 2-aminochrysene was incubated in the system described and in a similar system but containing either naphthalene or the potent inhibitor of microsomal "hydroxylases", SKF 525A. The correlation in Table 1 between the ability to degranulate microsomal membranes and carcinogenicity is complete for this system. A chemical substance is deemed carcinogenic if application *in vivo* can give rise to tumours, either at the site of application or in some distant tissue. Clearly not all of the "carcinogens" listed have been shown to induce hepatic neoplasms. Tissue specificity, however, is largely a matter of method of application, sex, age, dietary and hormonal status of the experimental animal (see, for example, ref. 11), so it may be justifiable to test all of these compounds *in vitro* in a hepatic system.

Table 2 shows the results of an experiment to confirm that the increase in apparent enzyme activity is due to membrane degranulation. Microsomal membranes pre-incubated as described were centrifuged over a layer of 2.0 M sucrose to

Table 2 Degranulation of Rough Surfaced Microsomal Membranes from Male Rat Liver caused by Incubation with the Substance indicated in the Presence of NADPH

Substance tested *	RNA removed from membrane (% control) measured directly	calculated †	Carcinogenicity
Naphthalene	0	0	Nil
Anthracene	0	0	Nil
2,7-Diaminofluorene	30	30	+
2-Aminochrysene	30	30	+
6-Aminochrysene	0	0	Nil
3-Aminopyrene	27	35	+
3,4-Benzpyrene	28	40	+
Carbon tetrachloride (15 µg ml. ⁻¹)	35	30	+

* Mixture of rough and smooth membranes incubated at 20° C for 2 h as described in footnote to Table 1, and centrifuged over a layer of 2.0 M sucrose for 4 h 120,000g to remove unbound ribosomes.

† Corresponding values calculated from the data summarized in Table 1²⁶.

remove unbound ribosomes. Membranes, both smooth and those containing bound ribosomes, float on the sucrose layer whereas any unbound ribosomal material will penetrate the sucrose and is thus separated from the membranes²⁵. The loss of RNA from the membranes is shown in Table 2 as a percentage of the RNA in control membrane samples. This correlates with the values calculated from data summarized in Table 1, confirming the original interpretation of the apparent enzyme activation results.

Table 3 Effect of Various Substances on the Carcinogen-induced Degranulation of Microsomal Membranes from Male Rat Liver *

Incubation †	Rates of degranulation			
	Benzanthraquinone	Dibenzpyrene	2-Aminochrysene	Carbon tetrachloride
Control	8	13	8	8
-NADPH	0	0	0	0
+ Naphthalene (10 µg ml. ⁻¹)	2	2	2	8
+ CO	0	0	Not tested	Not tested
+ SKF 525A (1 mM)	0	0	0	8
+ Oestradiol (1 µg ml. ⁻¹)	<0.5	0	<0.5	<0.5
+ Testosterone (1 µg ml. ⁻¹)	Not tested	9	Not tested	Not tested

* Measured as the rate of increase of apparent disulphide interchange enzyme activity.

† Conditions as described for Table 1 except additions where indicated.

The requirement for NADPH in this system suggested the involvement of the microsomal "hydroxylase" system. Table 3 shows preliminary results of an attempt to determine the nature of the activation of the substances tested in this system. The inhibition of the whole process (effected by the three polyaromatic substances) by CO and SKF 525A strongly suggests that cytochrome P450 is involved³², and the partial inhibition by an alternative hydroxylase substrate, naphthalene³³, is consistent with this view. Carbon tetrachloride, however, is not inhibited by any of these "hydroxylase" probes and this is consistent with the mechanism of activation described by Slater¹⁸ in which homolytic fission of the carbon tetrachloride molecule yields reactive radicals after interaction with the flavoprotein portion of the "hydroxylase". Cytochrome P450 is not required for this process. Oestradiol gives virtually complete protection against degranulation by all of the active compounds including carbon tetrachloride. This strongly suggests that the degranulation is caused by activated carcinogens attacking the oestradiol site on the membrane responsible for maintaining the integrity of the ribosome-membrane complex, although of course, as an alternative hydroxylase substrate, some inhibition by the steroid would be expected anyway. This scheme receives support from the experiment illustrated by Fig. 2. In this case membranes (mixed rough and smooth) were treated with the substances indicated and separated from unbound ribosomes in exactly the same way as described in the footnote to Table 2. They were then incubated with fresh polysomes and oestradiol. In these conditions the smooth membrane present should bind ribosomes with consequent time dependent loss of apparent enzyme activity, as indeed happens with the control membranes and those treated with naphthalene. The membranes treated with 3,4-benzpyrene, 3-aminopyrene or carbon tetrachloride, however, lose their activity only at the same slow rate as the control membranes without steroid. Two important conclusions can be drawn from this experiment:

first, the carcinogen-induced degranulation is operationally irreversible, and second, the carcinogens destroy the ability of smooth membranes to bind polysomes in the presence of oestradiol. It is also clear that the interactions of the carcinogens with the smooth membranes occur faster than carcinogen-induced degranulation.

Common Denominator for Carcinogens

Interference with membrane-ribosome association is thus a common property of the carcinogens tested. This is consistent with the findings of electron microscopists who have observed degranulation of rough surfaced endoplasmic membranes *in vivo* in tissues treated with a range of toxic agents in acute and carcinogenic situations^{18,32,33}. It is reasonable to suppose that the degranulating agents used in this study act on this system in exactly the same way as aflatoxin B₁, the effects of which are now fairly well characterized. Thus the carcinogens occupy or destroy a site on the membrane for steroid hormones. The specificity of the reaction is quite remarkable and well illustrated by the comparison between 2 and 6-aminochrysene. The innocuous 6-aminochrysene has no detectable effect on this system. Similarly the non-carcinogen benzo[b]chrysene is totally devoid of activity. None of the toxins tested, other than aflatoxin, has any action, either degranulating rough membranes or interacting with smooth surfaced membranes in such a way as to prevent polysome association, unless they are activated by a functional P450 complex. Even more remarkably, carbon tetrachloride degranulates rough membranes only after activation by the hydroxylase system.

It would be premature to claim that the action of the carcinogens discussed above is the common denominator of carcinogenic activity until a more extensive range of substances has been tested. The fact that the carcinogens interfere with a process controlled by the developmental hormones is consistent

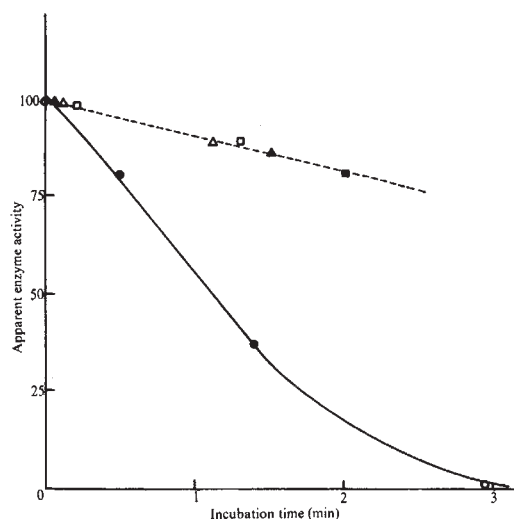


Fig. 2 The ability of pretreated smooth microsomal membranes to bind added ribosomes in the presence of oestradiol. Male microsomal membranes (about 5 mg protein ml.⁻¹) were pretreated, as described in the footnote to Table 2, with naphthalene (○); 3,4-benzpyrene (△); 3-aminopyrene (□); and carbon tetrachloride (▲); the membranes were incubated at 21° C with polysomes (c. 5 mg RNA ml.⁻¹) and oestradiol (2 µg ml.⁻¹) in a buffer containing 50 mM Tris, 25 mM KCl, 5 mM MgCl₂, and 250 mM sucrose, at pH 7.3, as described previously^{25,26}. A control incubation without steroid was also run (■). Pretreatment with benzpyrene, aminopyrene and carbon tetrachloride caused an increase in apparent enzyme activity measured at zero time as described in the text, so for convenience the apparent enzyme activity of the membranes in the incubations has been expressed as a percentage of the initial activity.

with the concept of cancer as a form of aberrant differentiation. It is also interesting that the interaction described here would, *in vivo*, seriously disturb the pattern of template RNA lifetime because the messenger is probably more stable if attached to a membrane. Degranulation could thus bring about the selective labilization of those same species of mRNA originally bound to the membrane which would be consistent with the hypothesis of malignancy proposed by Pitot³⁷.

There is one apparent exception to the correlation we describe. The hepatocarcinogen, ethionine, does not seem to interact with the membrane in the same way as the other carcinogens described here. Recent unpublished results in these laboratories have shown that the lesions produced by ethionine are in the ribosomes and that these are modified *in vivo* in such a manner as to prevent their association with untreated membranes. Hence the exception is more apparent than real, for the same system is disrupted.

Observations suggest that it is possible to devise an *in vitro* "screen" test for potentially carcinogenic substances, based on membrane-polysome association, which would enable a large number of compounds to be screened very rapidly. We do not suggest that such a test could replace *in vivo* testing completely but it could be of great value for rapidly and cheaply defining potential chemical hazards which would then need to be properly evaluated in the usual way.

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¹ Rusch, H. P., *Cancer Res.*, **14**, 407 (1954).

² Miller, J. A., and Miller, E. C., *Cancer Res.*, **25**, 1292 (1965).

³ Farber, E., *Cancer Res.*, **28**, 1859 (1968).

⁴ McLean, A. E. M., and McLean, E. K., *Brit. Med. Bull.*, **25**, 278 (1969).

⁵ Parke, D. V., and Williams, R. T., *Brit. Med. Bull.*, **25**, 256 (1969).

⁶ Irving, C. C., *J. Biol. Chem.*, **239**, 1595 (1964).

⁷ Arrhenius, E., *Cancer Res.*, **28**, 264 (1968).

⁸ Westrop, J. W., and Topham, J. C., *Biochem. Pharm.*, **15**, 1395 (1966).

⁹ Miller, J. A., Cramer, J. W., and Miller, E. C., *Cancer Res.*, **20**, 950 (1960).

¹⁰ Arrhenius, E., *Chem.-Biol. Interactions*, **1**, 361 (1970).

¹¹ Arcos, J. C., and Argus, M. F., *Adv. Cancer Res.*, **11**, 305 (1968).

¹² Lijinsky, W., Loo, J., and Ross, A. E., *Nature*, **218**, 1174 (1968).

¹³ Conney, A. H., *Pharmacol. Rev.*, **19**, 317 (1967).

¹⁴ Preussmann, R., Hodenberg, Avon, and Hengy, H., *Biochem. Pharm.*, **18**, 1 (1969).

¹⁵ Boyland, E., and Sims, P., *Biochem. J.*, **95**, 788 (1965).

¹⁶ Miller, J. A., and Gelboin, H., *Fed. Proc.*, **29**, 737 (1970).

¹⁷ Miller, E. C., and Miller, J. A., *Pharmacol. Rev.*, **18**, 805 (1966).

¹⁸ Slater, T. F., *Meth. Archieum. Exp. Pathol.*, **4**, 30 (1969).

¹⁹ Prodi, G., Rocchi, P., and Grilli, S., *Cancer Res.*, **30**, 1020 (1970).

²⁰ Brookes, P., *Cancer Res.*, **26**, 1994 (1966).

²¹ Roberts, J. J., and Warwick, G. P., *Intern. J. Cancer*, **1**, 179 (1966).

²² Gram, T. E., Rogers, L. A., and Fouts, J. R., *J. Pharm. Exp. Therap.*, **155**, 479 (1967).

²³ Scaife, J. F., *FEBS Lett.*, **12**, 143 (1971).

²⁴ Roy, A. K., *Biochim. Biophys. Acta*, **169**, 206 (1968).

²⁵ Williams, D. J., and Rabin, B. R., *FEBS Lett.*, **4**, 103 (1969).

²⁶ Sunshine, G. H., Rabin, B. R., and Williams, D. J., *Nature*, **230**, 133 (1971).

²⁷ Litwack, G., and Morey, K. S., *Biochem. Biophys. Res. Commun.*, **38**, 1141 (1970).

²⁸ Williams, D. J., Gurari, D., and Rabin, B. R., *FEBS Lett.*, **2**, 133 (1968).

²⁹ Epstein, S. S., Small, M., Falk, H. L., and Mantel, N., *Cancer Res.*, **24**, 855 (1964).

³⁰ Recknagel, R. O., *Pharmacol. Rev.*, **19**, 145 (1967).

³¹ Barnes, J. M., and Butler, W. H., *Nature*, **202**, 1016 (1964).

³² Orrenius, S., *J. Cell Biol.*, **26**, 713 (1965).

³³ Posner, H. S., Mitoma, C., and Udenfriend, S., *Arch. Biochem. Biophys.*, **94**, 269 (1961).

³⁴ Svoboda, D., and Higginson, J., *Cancer Res.*, **28**, 1703 (1968).

³⁵ Flaks, B., *Chem.-Biol. Interactions*, **2**, 129 (1970).

³⁶ Butler, W. H., *Amer. J. Pathol.*, **49**, 113 (1966).

³⁷ Pitot, H. C., *Persp. Med. Biol.*, **8**, 50 (1964).

³⁸ Blyth, C. A., Freedman, R. B., and Rabin, B. R., *Nature*, **230**, 137 (1971).

³⁹ Rabin, B. R., Sunshine, G. H., and Williams, D. J., in *Biochem. Soc. Symp.*, No. 31 (edit. by Smellie, R. M. S.), 203 (Academic Press, New York and London, 1970).

⁴⁰ Roobol, A., and Rabin, B. R., *FEBS Lett.*, **14**, 165 (1971).

Nonbiotic Origin of Optical Activity

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Asymmetry in a planetary system, with consequent effects on available light, could have been involved in the origin of optically active chemicals.

THE origin of optically active compounds has been a question of debate since the days of Pasteur and among the various explanations that have been postulated are (a) autocatalytic propagation of asymmetry by an individual molecule that happened to be first in a D-L-pair, (b) photochemical reactions with chiral or circularly polarized light¹ produced by reflexion or magnetic fields, (c) interaction with asymmetric minerals like quartz and (d) inherent energetic difference between antipodal molecules perhaps connected with nuclear asymmetry as manifested in radioactive β -decay².

All these hypotheses except the last rest on the assumption

of local and/or occasional excess of one of the antipodal forms of an asymmetric agent. In the absence of any explanation as to how such excesses could arise over sufficiently wide areas and periods of time, they therefore reduce to hypotheses about chance occurrences³. In the following a possible relationship between asymmetry in a planetary system and molecular asymmetry is discussed.

It is well known that magnetic fields can rotate the plane of polarization of plane polarized light travelling through a medium (Faraday effect)⁴. The rotation R , which is positive for clockwise rotation viewed in the direction of light travel, is given by: $R = V/H \cos \phi$ where V is the so called Verdet constant characteristic of the medium at a given temperature and wavelength, H is the strength of the magnetic field, I is the distance of light travel and ϕ is the angle between the lines of force of the field and the direction of light propagation. ϕ changes from zero for the parallel to 180° for the antiparallel case.

The equation shows that R will be zero when $\phi = 90^\circ$ and

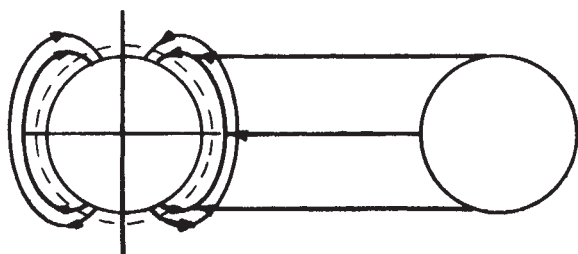


Fig. 1 A planet orbiting a "sun" as described in point (1) in the text. The planet is represented by the solid circle to the left and the "sun" is to the right. The light rays are symbolized by straight arrows. The dashed circle represents the "boundary" between atmosphere and space. The curved arrows represent the lines of force of the magnetic field going from "south" to "north".

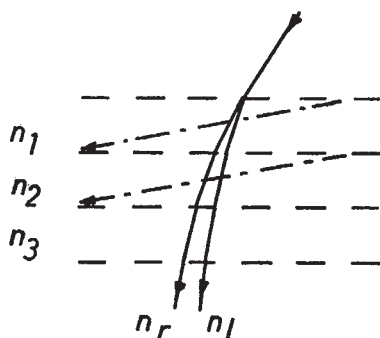


Fig. 2 The enhancement by a refractive index gradient of the separation in a magnetic field of two oppositely circularly polarized light beams. The beams are represented by solid arrows, n_r being the refractive index of the right circularly polarized beam and n_l that of the oppositely polarized beam. The broken line arrows represent the lines of force of the magnetic field. n_1 , n_2 and n_3 represent the downwards increasing refractive indices for the light in the absence of the field in three different layers of matter.

will change sign when the direction of propagation of light is reversed. The rotation is equivalent to a speed or refractive index difference between the equally strong right and left circularly polarized beams into which the plane polarized one can be split. This is given by: $R = \pi/\lambda(n_l - n_r)$ where n_l and n_r are the refractive indices of the left and right handed beams respectively and λ the wavelength. The right handed beam is as much accelerated as the left handed beam is retarded.

Light passing non-perpendicularly through a boundary from one region to another (Fig. 2) with a different refractive index will be refracted. A magnetic field non-perpendicular to the direction that the light would take in the second region will cause a refractive separation of right and left circularly polarized light, because the refractive indices for the two circularly polarized beams will differ as a result of the field.

All this can be applied to a spherical planet orbiting a large light-emitting central body, the "sun". The dimensions of the two bodies are taken to be small relative to the distance between them. The planet has an atmosphere with an increasing density and refractive index on going towards its centre. Furthermore, it has an axial rotation and a magnetic dipole field, the axis of which coincides with the rotational one. The dipole midpoint is in the planetary centre. The following is a discussion of the effect on one of the plane polarized components of which an unpolarized beam is composed.

(1) Let the equatorial plane be parallel to the orbital one (Fig. 1). On the upper part of the "northern" hemisphere the magnetic field then has a component parallel to the light rays from the "sun". Here the rays travel almost parallel to

the atmospheric "boundary". A refractive separation of right and left circularly polarized light will therefore arise. For a positive Verdet constant the latter beam will be retarded and refracted down more steeply into the atmosphere. More of the right handed light will therefore disappear out into space and the planetary atmosphere will here be enriched in the oppositely polarized light. The refractive index gradient should multiply the separation effect (Fig. 2). (Apart from changes in direction and strength of the magnetic field that the travelling light rays will encounter because of the dipolar nature of the field this picture should remain qualitatively true.) In the lower part of the "southern" hemisphere the situation will clearly be the reverse.

In the region where the light comes in almost at right angles to the atmosphere, little refraction will occur. Symmetry reasoning shows that no net excess of one circularly polarized light form and hence of photochemically produced asymmetric material should be found over the planet taken as a whole.

The system is symmetric, for it is superimposable on its mirror image. (The mirror image of a magnet parallel to a mirror has the poles inverted.)

(2) Let the planet have its equatorial plane tilted at an angle α with respect to its orbit (Fig. 3). The magnetic field in the "southern" hemisphere and also somewhat "north" of the equator now has a component antiparallel to the light. On the upper part of the "northern" hemisphere the situation is more complicated. In certain latitudes between "pole" and "polar-circle" the field has a component parallel to the light during the "day". "Twelve hours" later the opposite is true. Thus here the effects during "day" and "night" will oppose each other. A small area immediately surrounding the "north" pole, where the field-lines are almost parallel to the planetary axis, constitutes an exception to this, however. In this area a component of the field will be parallel to the rays all the time. It is difficult to say precisely which effect should predominate on the northern hemisphere as a whole, for the effect will depend on the magnetic "inclination" angle and hence on the magnetic dipole length in relation to the "dimensions" of the atmosphere.

When α is close to 90, the light in the polar region facing the "sun" will enter the atmosphere almost perpendicularly and refraction should be unimportant. In the equatorial zone with the rays coming in practically tangential to the atmosphere and antiparallel to the field the separation will be at a maximum, with enrichment in the right handed component. Thus in this situation enrichment of light circularly polarized in the same sense will occur at the equator and a bit north of it. Furthermore, for an α value of 90, even the "southern" hemisphere will be reached by some light that has been refracted by the atmosphere. This amount of light reaching the "southern" hemisphere during "summer" in the "northern" hemisphere will of course be smaller than the amount reaching the "south" during "summer" there. At the "equinoxes", that is, when "day" and "night" are equally long, the situation will be as under point (1). For a circular orbit therefore one hemisphere will have a "yearly" net excess of one circularly polarized light form while the other will have an equal excess

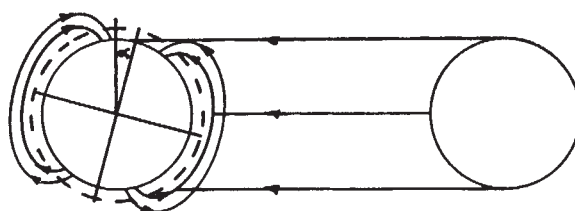


Fig. 3 A planet with its equator tilted at an angle α relative to its orbit as described under point (2) in the text. Everything else is as in Fig. 1.

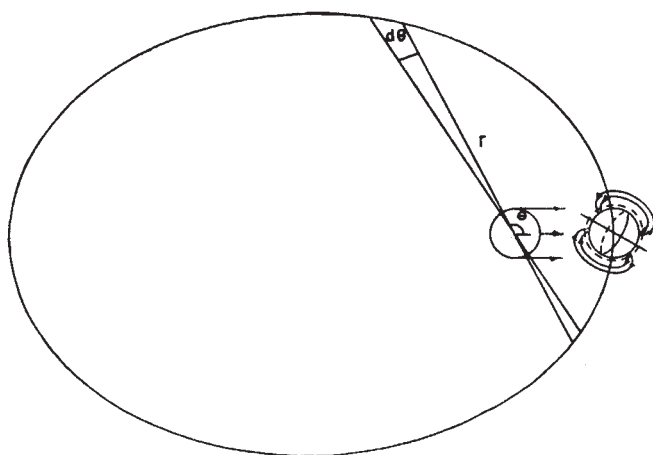


Fig. 4 A planet with tilted equator and elliptical orbit as described under point (3) in the text. The ellipse represents the orbit. θ is the angle between radius vector (as defined in the text) for any given position and the position closest to the "sun". The equator is seen in perspective. Everything else is as in Fig. 1.

of the opposite form. No net effect should thus result counted over the whole planet and the whole year.

In any given position in the orbit, the system is non-superimposable on its mirror image and is asymmetric. "Half a year" later the mirror image turns up, however. There is thus symmetry when the whole "year" is considered.

(3) Let the orbit be elliptical (Fig. 4). When the "sun" is large in comparison with the planet, the former is located in one focal point of the orbit. Kepler's second law states that radius vector r from the "sun" to the planet always sweeps out the same area in a given time interval. The area swept out when the planet moves through the angle $d\theta$ is $rd\theta$. The time required is thus proportional to $rd\theta$. The intensity of a light source is proportional to $1/r^2$. The total light energy falling on the planet then obviously is proportional to the product of intensity and time, that is $d\theta/r$. With polar coordinates as in Fig. 4 the equation of the ellipse is

$$r = \frac{b^2}{a + c \cos \theta}$$

where $2a$ is the length of the major, $2b$ that of the minor axis and $2c$ is the distance between the focal points. Integration gives

$$\int_{\theta_1}^{\theta_2} \frac{d\theta}{r} = \frac{a}{b^2} (\theta_2 - \theta_1) + \frac{c}{b^2} (\sin \theta_2 - \sin \theta_1)$$

If $\theta_2 - \theta_1 = \pi$ it follows that the amount of light received by the planet is

$$\frac{a}{b^2} \pi - \frac{2c}{b^2} \sin \theta_1$$

when it moves between θ_1 , and $\theta_1 + \pi$ and $0 < \theta_1 < \pi$, but is

$$\frac{a}{b^2} \pi + \frac{2c}{b^2} \sin \theta_1$$

when it continues from $\theta_1 + \pi$ to θ_1 .

We now consider the amounts of light that the two hemispheres get during their respective "summers". The "seasons" will change between "summer" and "winter" at the "equinoxes". The latter holds, where a line through the "sun", parallel to the intersection between the equatorial and orbital planes, cuts the orbit. Clearly, the "equinoxes" are π radians apart and the hemisphere that has "summer" when the planet

is close to the "sun" will get most light in accordance with the integration above. (A pronounced example is Mars.)

As discussed in point (2), for a large α , one form of chiral light predominates in the equatorial region when one hemisphere has "summer" and vice versa. Given these "seasonal" excesses of the respective circularly polarized light forms, the light form that is in excess, when the hemisphere that receives most light, has "summer" should predominate on the planet as a whole over a "year". Thus the photochemically produced materials of the enantiomeric forms predominating when this hemisphere has "summer", will likewise predominate. When the intersection between orbit and equator is parallel to the major axis (that is $\theta_1 = 0$) "seasons" will be equally long on the two hemispheres and they will also have equal amounts of light energy.

The system is asymmetric in each point in the orbit and also over a whole "year" when $\alpha \neq 0$. This can be deduced as in the previous cases. The magnitudes of such effects are difficult to estimate and would undoubtedly be small on the present Earth. The small values, for gases, of the Verdet constants (generally higher, however, at shorter wavelengths where light is photochemically more effective), the thinness of the atmosphere and ensuing shallowness of the refractive index gradient, the weakness of the magnetic field, the small angle between the equator and the elliptic, and most of all the absence of a large eccentricity of the present orbit should all cause only small effects. Going back many billions of years the situation could, however, have been quite different in all these respects. Furthermore, there is at present no general way of telling theoretically how much chemical asymmetry a given photochemical reaction with circularly polarized light will produce⁵.

With roughly the same reasoning as above, one could visualize similar effects that could cause net asymmetry on an entire planet. Take, for example, a rotating "sun" that possesses a magnetic field coinciding with its axis and has its equator tilted relative to the orbit of a planet. If the planet has an elliptical orbit, unequal amounts of light from the two hemispheres of the "sun", will fall on it. Faraday effects in the "solar" atmosphere should work in the same way as described above for planets. The net result should be excess of one chiral form of light on the planet even without planetary atmosphere, magnetic field, and tilting of the planetary axis, all in analogy with previous reasoning. The Zeeman effect, which is closely related to the Faraday effect, could work in the same overall way.

Some factors which could increase the differences in amounts of enantiomeric molecular species when the antipodes of the starting material are already present in unequal amounts would be many consecutive reactions, where the asymmetric force, that is the asymmetric light, intervened at most individual steps (compare Harada where the case for presumed inherent antipodal energy differences is treated). If a sufficiently large number of steps are involved, this might give rise to large differences in amounts of enantiomeric end-products, even when the initial difference is small. A further case would be one in which many asymmetric molecules react with each other in many consecutive reactions. Diastereomeric discrimination at each step might here add up to large differences in the end. An attempt at making a quantitative estimate of the effects for various hypothetical "primitive earth" conditions by numerical treatment with a computer is being undertaken.

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¹ Kuhn, W., and Braun, E., *Naturwissenschaften*, **17**, 227 (1929).

² Ulbricht, T. L. V., *Quart. Rev.*, **13**, 48 (1959).

³ Harada, K., *Naturwissenschaften*, **57**, 114 (1970).

⁴ Waring, C. E., and Custer, R. L., in *Techniques of Organic Chemistry* (edit. by Weissberger, A.), **1**, Part 3, 2497 (Interscience, New York, 1960).

⁵ Hallas, G., *Organic Stereochemistry*, 56 (McGraw-Hill, London, 1965).

LETTERS TO NATURE

PHYSICAL SCIENCES

Heterocyclic Compounds indigenous to the Murchison Meteorite

THE origin of purines and pyrimidines is of prime importance in chemical evolution. Several pathways have been proposed for their laboratory synthesis in simulated primitive Earth conditions¹. There is only one report, however, on their possible occurrence in carbonaceous chondrites, which are presumed to be representative of primordial cosmic material². We thus undertook a search for heterocyclic compounds using a sample of the Murchison meteorite, a type II carbonaceous chondrite. We found 4-hydroxypyrimidine and two of its methylsubstituted isomers. We did not find any purines or triazines.

We chose the Murchison meteorite for analysis since recent work on the amino-acids³ and hydrocarbons⁴ in this particular chondrite showed clearly that contamination was not a serious problem. The type and distributions of organic compounds isolated suggested a random (abiogenic) synthesis.

A 5.1 g portion of the pulverized meteorite was taken from the same batch used in earlier studies on amino-acids³. The sample was refluxed in water for 24 h, supernatant was recovered and the precipitate refluxed in 88% formic acid for 24 h. Water and formic acid extracts were each concentrated and each mixed with 750 mg of an extensively washed 1:1 mixture of Norit charcoal and celite 545 for 3 h⁵. These mixtures were washed with three 10 ml. volumes of water. Adsorbed material was eluted by heating mixtures in 88% formic acid for 2 h at 40° C. The clear yellow-brown supernatants were concentrated under dry, filtered N₂ gas to 8 ml. One ml. aliquots were taken to dryness and the trimethylsilyl derivatives were prepared⁶ for gas chromatography-mass spectrometry (GC-MS).

A procedural blank yielded several easily resolvable trace components, labelled B in Fig. 1. The charcoal-celite has been tested⁵ and is capable of recovering quantitatively from dilute solutions a wide variety of purines, pyrimidines, some amino-acids, triazines, and other non polar aromatic heterocyclic compounds.

The mass spectrometer total ion monitor trace (Fig. 1) from combined low resolution GC-MS of the formic acid extract of the meteorite was equivalent to the GC trace and presents a number of well resolved major and minor peaks. Those numbered 1-3 are heterocyclic; other major peaks seem to represent amino-acid derivatives, or linear and branched polymers of $(-\text{CH}_2\text{CH}=\text{N}-)_n$ and will be dealt with in future publications.

Low resolution mass spectra taken of compounds 1-3 along with spectra of standard compounds are presented in Fig. 2. Combined GC-high resolution mass spectrometry on an aliquot of the same sample permitted determination of elemental composition of major fragment ions for unknowns 1 and 2. Insufficient sample size and derivative instability militated against our obtaining meaningful high resolution data for unknown 3.

For compound 1 we found: at m/e 153, the trimethylsilyl derivative M-15 peak, $\text{C}_6\text{H}_9\text{N}_2\text{O}_1\text{Si}_1$ (153.04841 calculated, 153.04982 found, +1.41 mmu error), at m/e 99, $\text{C}_4\text{H}_7\text{O}_1\text{Si}_1$ (99.02662 calculated, 99.02670 found, -0.08 mmu error). For compound 2 we found: at m/e 101, $\text{C}_4\text{H}_9\text{O}_1\text{Si}_1$ (101.04226 calculated, 101.04630 found, -4.04 mmu error).

The low and high resolution mass spectral data suggest that compound 1 is 4-hydroxypyrimidine. Standard 4-hydroxypyrimidine has an identical GC retention time and yielded an identical low resolution mass spectrum (Fig. 2).

An unambiguous identification requires that sample and standard compounds possess identical GC retention times and mass spectra. Although these criteria were met for compound 1, exact standards were not available for compounds 2 and 3. Compound 2, however, seems to be 4-hydroxymethylpyrimidine. Its mass spectrum (Fig. 2) shows a major fragment ion at m/e 101, explicable by bond cleavage at the number 4 carbon, accompanied by a hydrogen transfer to give $\text{C}_4\text{H}_9\text{O}_1\text{Si}_1$.

Compound 3 has a similar GC retention time and a similar mass spectrum to that of the related standard 2-hydroxy-4-methylpyrimidine (Fig. 2). Unknown and standard mass spectra differ only in the m/e 99 (unknown) and m/e 100 (standard) peaks. This unknown seems to be a methyl isomer of 4-hydroxypyrimidine. Exact location of the methyl group is not possible with these data. We can exclude the N-methyl locations since the total number of rings plus double bonds for this compound is 4. Ring position 5 is excluded since the major fragment ion at m/e 99 requires a hydrogen at this location. This unknown we tentatively identify as 4-hydroxy-2- or -6-methylpyrimidine.

The water extract of the meteorite contained these same three compounds in somewhat greater abundance as well as other members of the hydroxypyrimidine series. Based solely on low

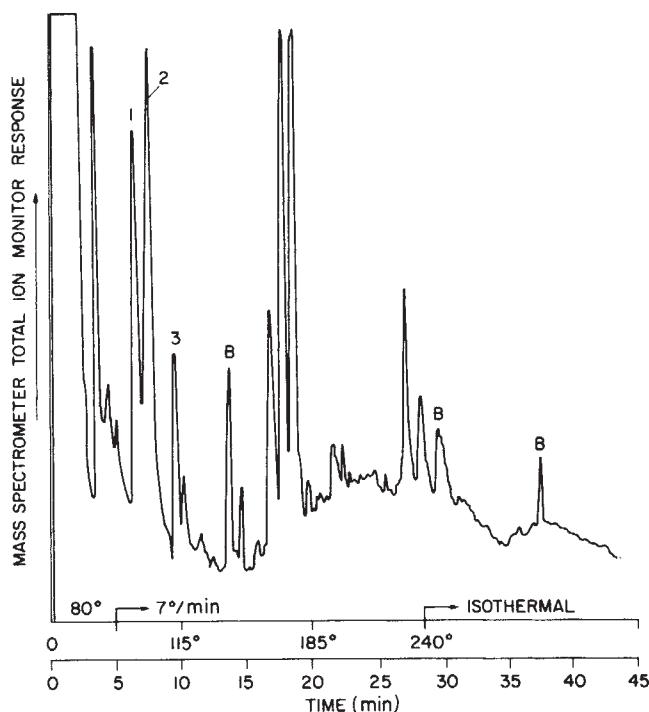


Fig. 1 Total ion monitor trace; combined gas chromatography-mass spectrometry of trimethylsilyl derivatives of the Murchison meteorite formic acid extract. Analytical conditions were: 3.3 m x 2 mm inner diameter glass 3% OV-17 column in a Loenco 160 x gas chromatograph; helium flow 12 ml. min⁻¹; column maintained at 80° for 5 min, then temperature programmed at 7° min⁻¹ to 240°. Membrane separator at 190°; Consolidated Electrochemicals 21-491 mass spectrometer, scan rate of 4 s decade⁻¹.

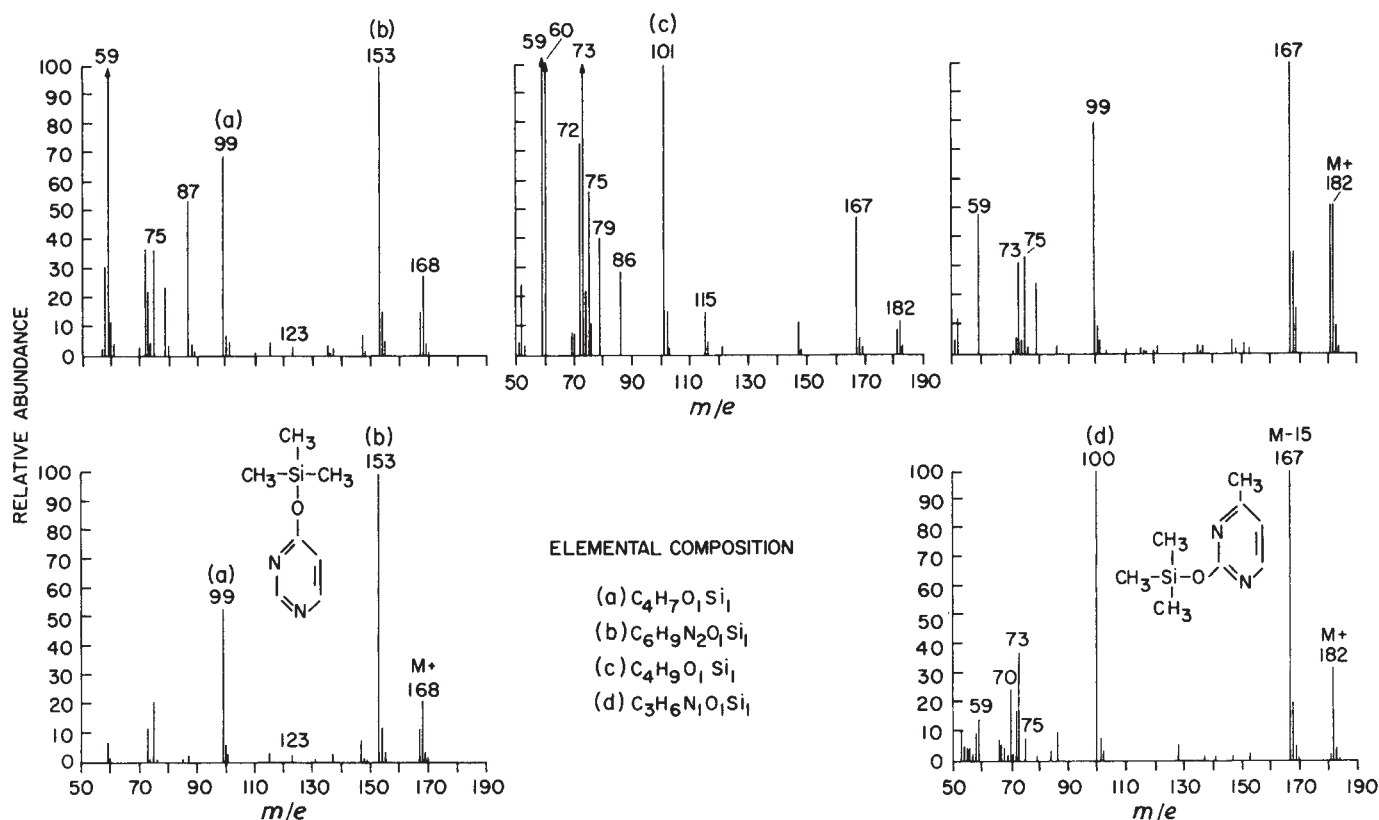


Fig. 2 Top row, left to right, low resolution mass spectra taken of peaks 1, 2, and 3 of Fig. 1. Standard 4-hydroxypyrimidine and related standard 2-hydroxy,4-methylpyrimidine mass spectra obtained by GC-MS using the Consolidated Electrodynamics 21-110 mass spectrometer in the low resolution mode. Elemental compositions of marked peaks were determined using combined GC-MS and the 21-110 mass spectrometer photoplate detector.

resolution mass spectra, we tentatively identify: (a) a 5,6-dehydro-5,6-dihydroxypyrimidine, (b) a 4-hydroxy-2-methyl-2-amino-quinazoline, and (c) a 4-hydroxy-2-methyl-6-carboxypyrimidine. There was insufficient material (less than 100 ng) to permit high resolution mass spectrometry of these compounds and standards were not available. The water extract data indicated that the hydroxy-substituted compounds found were not the result of acid hydrolysis of a pre-existing amino function.

Standard purines, pyrimidines, triazoles, triazines and pyrazinols were taken through the entire GC-MS low resolution analytical scheme, which is capable of detecting unambiguously 50–70 ng of each as its trimethylsilyl derivative. Although specifically sought, no trace of adenine, guanine, xanthine, hypoxanthine, cytosine, uracil, thymine, amino-sym-triazine, amino triazole, melamine, ammelamine, cyanuric acid, or ammelide was found. These negative observations support other work^{3,4} which argues for the absence of biological or plasticizer contamination of this meteorite.

The amounts of recovered pyrimidines were estimated with an uncertainty of 30–50% by GC flame ionization detector response. We calculate $6 \mu\text{g g}^{-1}$ for 4-hydroxypyrimidine, $7 \mu\text{g g}^{-1}$ for 4-hydroxymethylpyrimidine, and $3 \mu\text{g g}^{-1}$ for 4-hydroxy-2(or 6)-methylpyrimidine.

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- ¹ Ponnamperuma, C., *Quart. Rev. Biophys.* (in the press).
- ² Hayatsu, R., *Science*, **146**, 1291 (1964).
- ³ Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperuma, C., Kaplan, I. R., and Moore, C., *Nature*, **228**, 923 (1970).
- ⁴ Pering, K., and Ponnamperuma, C., *Science* (in the press).
- ⁵ *A Search for Carbon and its Compounds in Lunar Samples from Mare Tranquillitatis* (edit. by Kvenvolden, K., and Ponnamperuma, C.) (NASA SP-257, 1970).
- ⁶ Gehrke, C. W., and Ruyle, C., *J. Chromatog.*, **38**, 473 (1968).

Cosmological Upper Limit on the Time Variation of G

RECENT results of Shapiro *et al.*¹ based on an analysis of radar echo time delays have set an experimental upper limit on the possible time variation of the gravitational constant as $|\dot{G}/G| < 3 \times 10^{-10} \text{ yr}^{-1}$. Theoretical calculations by Dicke² for zero pressure Friedman-type cosmologies according to the Brans-Dicke (BD) theory³ yield $|\dot{G}/G| \approx 10^{-11} \text{ yr}^{-1}$ for flat space and $|\dot{G}/G| \approx 3 \times 10^{-11} \text{ yr}^{-1}$ for closed space. Although this time variation of G is extremely small at the present epoch, Dicke has shown⁴ that there exist early epoch solutions (types 2 and 3) for which the energy density associated with the time variation is much greater than the matter energy density. (Dicke also finds a solution (type 1) which is not scalar dominated.)

My new results are based on exact cosmological solutions^{5,6} valid for all epochs and extend Dicke's theoretical bound on $\dot{G}/G$ to the case of pressure-filled cosmologies in flat space. It is

also shown that all Friedman-type BD cosmologies are scalar dominated at early epochs.

The flat space cosmologies yield the following exact expression which essentially gives the ratio of matter energy density (equation of state: $p = \varepsilon \rho c^2$) to scalar field energy density ($\rho_\lambda = (2\omega + 4/32\pi \varepsilon_0) \dot{\Lambda}^2$; $\Lambda = \ln \lambda$) as

$$\frac{8\pi\phi_0^{-1}\rho_m}{\Lambda^2} = \frac{h(d+h)}{24(1-\varepsilon)} \frac{z^2-1}{[(1-3\varepsilon)z+e_1]^2} \quad (k=0) \quad (1)$$

where the parameter z is related to the cosmic time t by the quadrature

$$t(z) = \frac{4D^3}{e_1 B} \int dz (z^2-1)^{(d-h)/2h} \left[\frac{z-1}{z+1} \right]^{e_1/h} \quad (2)$$

where $d = (6\omega + 9)(1 - \varepsilon^2) + (1 - 3\varepsilon)^2$, $h = (6\omega + 9)(1 - \varepsilon)^2 - (1 - 3\varepsilon)^2$, and $e_1 = (6\omega + 9)^{1/2}(1 - \varepsilon)$; B and D are constants of integration related to the initial conditions. Curved space solutions have been found only in the case $\varepsilon = 1/3$; they yield⁵

$$\frac{8\pi\phi_0^{-1}\rho_m}{\Lambda^2} = \phi_0^{-1} q B'^{-2} a^2 \quad (k = 0, \pm 1) \quad (3)$$

where a is the expansion parameter, $q = \rho_{m0} a_0^4$, $B' = a_0^3 \dot{\Lambda}_0$, and the subscript 0 denotes the present epoch.

From equation (1) it is evident that for late epochs ($z \rightarrow \infty$) the ratio ρ_m/ρ_λ approaches a constant for $\varepsilon \neq 1/3$ and in the same limit equation (3) shows that for $\varepsilon = 1/3$ the ratio becomes infinite in open spaces and constant in a closed space.

For early epochs ($z \rightarrow 1$) the curvature term contributes negligibly to the dynamics of the cosmology, so that it may be neglected there and the flat space results are valid. Inspection of equation (1) shows that the ratio ρ_m/ρ_λ approaches zero in this limit independent of ε . (The time behaviour, however, does depend on ε .) It is also clear that this dimensionless ratio must also be independent of the particular unit formalism used^{7,8}. Thus the dominance of ρ_λ over ρ_m as $t \rightarrow 0$ is a general feature of the Friedman cosmologies, and the scalar field, if it exists, must have had a crucial role in the initial phases of the universe.

In the case of the flat space cosmologies with $\varepsilon \neq 1/3$, equation (1) gives a relation between $\dot{\Lambda}$ and ρ_m at the present epoch. Another expression which results from evaluating a first integral⁶ of the cosmological equations at the present epoch yields a relation between $\dot{\Lambda}_0$ and t_0 . As the Hubble age⁹ $t_H = 3dt_0/(d+h)$ is the observable quantity, it is taken as the independent parameter and the following expressions result⁶

$$\begin{aligned} \dot{\Lambda}_0 &= \frac{12(1-3\varepsilon)}{(d+h)} t_H^{-1} \\ \rho_{m0} &= \frac{3}{8\pi\varepsilon_0} \frac{2\omega+4}{2\omega+3} \frac{2h}{(d+h)(1-\varepsilon)} t_H^{-2} \end{aligned} \quad (k=0) \quad (4)$$

For $\varepsilon = 0$ equations (4) agree with those of Brans and Dicke³; care must, however, be taken in comparing the two results because they are expressed in different unit-formalisms. For $\omega \approx 5$ and $\varepsilon = 0$ (ref. 10) and the presently accepted value of $t_H = 4 \times 10^{17}$ s, one finds $\dot{\Lambda}_0 \approx 10^{-11} \text{ yr}^{-1}$ and $\rho_{m0} \approx 10^{-29} \text{ g cm}^{-3}$. The above value for $\dot{\Lambda}_0$ is also an upper limit (in flat space) which decreases with increasing ε , whereas ρ_{m0} is a rather insensitive function of ε . Although there are no corresponding results available for curved spaces, it seems unreasonable to expect that the introduction of pressure can do anything but decrease the upper bound on $\dot{\Lambda}_0$ even in curved space. For the scalar field pressure, which is proportional to $\dot{\Lambda}^2$, augments the matter pressure at any given epoch. Thus the amount of scalar pressure required to expand against gravitational attraction of matter and reach the set of initial conditions at the present epoch will necessarily be smaller when matter pressure is present than when it is not. The corresponding value of $\dot{\Lambda}_0$ will therefore always be less than that required by the pressure free cosmology.

If observations are continued for another five years¹, the experimental upper limit will approach the pressure-free closed space value of $|\dot{G}/G| \approx 3 \times 10^{-11} \text{ yr}^{-1}$. My results show explicitly that the introduction of pressure decreases the theoretical upper bound in the case of flat space and arguments have been put forward that this is also true for curved spaces. If the chief component of cosmic matter is in the form of a radiation gas (for example, neutrinos) a relation (compare equation (4)) between $\dot{\Lambda}_0$ and t_H does not exist. Matter and radiation are decoupled in this case and almost any value of $\dot{\Lambda}_0$ can be made consistent with the present observables. Thus in principle the limits placed on the variation of G can be translated into limits on the equation of state for the expanding cosmic matter. Although all Friedman models display an extremely weak variation of G at the present epoch, the associated scalar energy density is dominant for early epochs. Thus the scalar field may play an important role in collapse situations in general.

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- ¹ Shapiro, I. I., Smith, W. B., Ash, M. D., Ingalls, R. P., and Pettengill, G. H., *Phys. Rev. Lett.*, **26**, 27 (1971).
- ² Dicke, R. H., *Science*, **138**, 653 (1962).
- ³ Brans, C., and Dicke, R. H., *Phys. Rev.*, **124**, 925 (1961).
- ⁴ Dicke, R. H., *Astrophys. J.*, **152**, 1 (1968).
- ⁵ Morganstern, R. E., *Phys. Rev. D* (in the press).
- ⁶ Morganstern, R. E., *Phys. Rev. D* (in the press).
- ⁷ Dicke, R. H., *Phys. Rev.*, **125**, 2163 (1962).
- ⁸ Morganstern, R. E., *Phys. Rev. D* (in the press).
- ⁹ Morganstern, R. E., *Phys. Rev. D* (in the press).
- ¹⁰ Dicke, R. H., and Goldenberg, H. Mark, *Phys. Rev. Lett.*, **18**, 313 (1967).

Acoustic Microscope operating at 100 MHz

THE concept of acoustic microscopy is not new¹, but the means for constructing a working device are only now becoming available^{2,3}. As acoustic microscopy relies on mechanical vibration, rather than electromagnetic radiation, it may be possible to visualize features of a specimen which go undetected by conventional methods. The lower propagation velocity of sound compared with that of light, in all known materials, results in a corresponding reduction in wavelength for a given frequency of excitation. The theoretically attainable resolution with acoustic microscopy is limited by the angular aperture and the wavelength in a manner entirely analogous to the optical situation. Present ultrasonic technology limits the upper frequency attainable to about 10 GHz which corresponds to a wavelength of 3000–6000 Å in most solids and 1000–2000 Å in most liquids.

In this communication we report on initial experiments with an acoustic microscope operating at 100 MHz with a resolution of the order of 25 μm. The basic principle of operation is similar to that used earlier in an experiment with acoustic holography at lower frequencies⁴ and is based on a technique used by Adler *et al.*⁵ for the visualization of acoustic surface waves. A detailed analysis may be found in ref. 6. A simplified diagram of the experimental configuration is shown in Fig. 1. An ultrasonic transducer launches a 100 MHz plane wave through a specimen suspended in water. The transmitted sound, which carries spatial information about the specimen, strikes a plastic mirror oriented at 45° with respect to the nominal direction of sound travel and causes a dynamic ripple pattern on the mirrored surface. The ripples cause periodic

angular deflexion of a focused laser beam reflected off the mirror. This angularly modulated, reflected light beam gives rise to an electrical signal in a photodiode when half the beam is intercepted by a knife edge.

The electrical signal is directly proportional to the angular displacement of the mirrored surface and hence to the local sound pressure amplitude. To achieve real time operation, the focused light beam is made to scan rapidly over a selected area of the mirror by means of two acousto-optic light deflector cells⁷. The scan of the light beam is synchronized with that of a conventional television monitor. The lens shown in Fig. 1 serves to keep the exit pupil of the laser scanner focused on the knife edge during scanning of the beam. If the 100 MHz signal from the photodiode is rectified and applied to the television monitor, a magnified picture of the sound field at the mirror appears on the screen. In an alternative mode of operation the signal from the photodiode is first applied to a phase detector where it is compared to an electronic reference signal. In that case the television screen shows an acoustic hologram rather than a conventional sound picture.

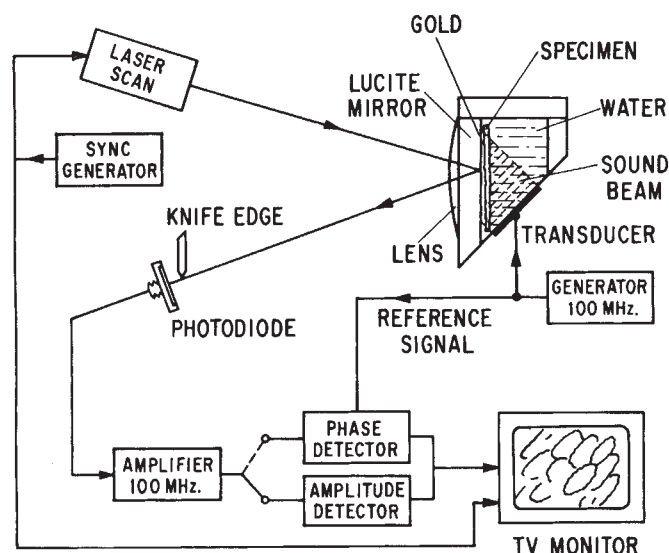


Fig. 1 Experimental configuration.

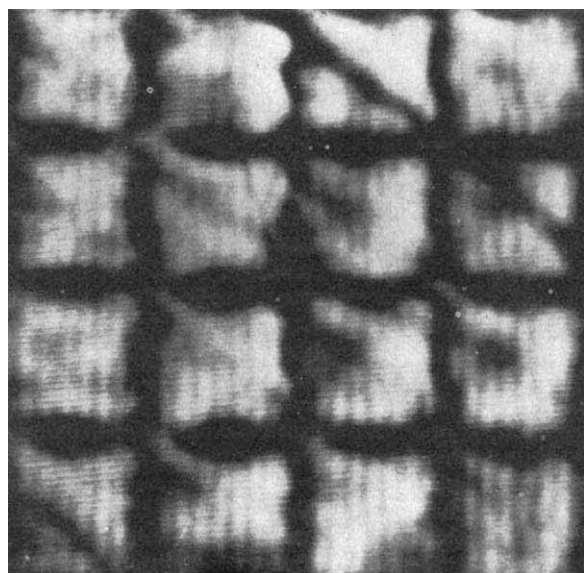


Fig. 2 Acoustic image of mesh composed of 25 μm diameter wires spaced on 100 μm centres.

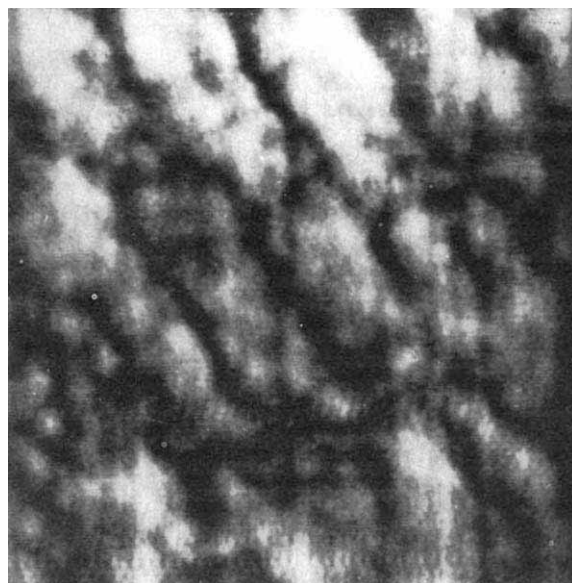


Fig. 3 Acoustic image of onion skin at the same magnification as Fig. 2.

In our experiment the laser beam is focused to a spot size of about 12 μm and scans an area of approximately $1 \times 1 \text{ mm}$. The magnification from mirror to television screen is approximately 400. The sound wavelength in the water is 15 μm . Fig. 2 shows an acoustic picture of our test object, a grid of fine wires with 100 μm centre spacing and 25 μm diameter. Note that the resolution is better than 25 μm , corresponding to 1.7 wavelengths. Also in Fig. 2, faint vertical lines can be seen which result from the presence of a spurious reference signal.

Although it is possible to suppress this signal, the presence of these lines actually shows the holographic capability of the technique. In Fig. 3 is shown what we believe to be the first picture of actual biological cell structure taken by an acoustic microscope. This picture shows the cell walls in a piece of onion skin and, although these initial pictures are somewhat crude, we believe they show the technological feasibility of this instrument. We plan to determine its usefulness by visualizing a series of different specimens and comparing them with the corresponding optical microscope pictures. Based on results with different methods of acoustic visualization at lower frequencies^{8,9} we believe that the acoustic microscope, when perfected, will be a valuable addition to more conventional instruments.

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- ¹ Dunn, F., and Fry, W. J., *J. Acoust. Soc. Amer.*, **31**, 632 (1959).
- ² Auld, B. A., Addison, R. C., and Webb, D. C., *Acoustical Holography*, 2 (edit. by Metherell, A. F., and Larmore, L.), Ch. 10 (Plenum Press, New York, 1970).
- ³ Korpel, A., and Kessler, L. W., *Acoustical Holography*, 3 (edit. by Metherell, A. F.), Ch. 3 (Plenum Press, New York, 1971).
- ⁴ Korpel, A., and Desmares, P., *J. Acoust. Soc. Amer.*, **45**, 881 (1969).
- ⁵ Adler, R., Korpel, A., and Desmares, P., *IEEE Trans. on Sonics and Ultrasonics*, **SU15**, 157 (1968).
- ⁶ Whitman, R. L., and Korpel, A., *Appl. Optics*, **8**, 1567 (1969).
- ⁷ Korpel, A., Adler, R., Desmares, P., and Watson, W., *Appl. Optics*, **5**, 1667 (1966).
- ⁸ Korpel, A., *Intern. J. of Nondestructive Testing*, **1**, 337 (1970).
- ⁹ Landry, J., Powers, J., and Wade, G., *Appl. Phys. Lett.*, **13**, 186 (1969).

Mercury in North-Eastern Atlantic Ocean Water

Few measurements have been made of the concentration of mercury in sea water and there are considerable differences in the values reported. High values of 1.6 to 3.6 μg total Hg/l. for oxidized samples from Minamata Bay, Japan¹, probably reflect the serious pollution of that area². Ocean water from the north-western Pacific has been reported³ to contain from 0.06 to 0.27 μg /l., with the highest concentrations in deep water. These measurements were made spectrophotometrically with dithizone, which was also used, in different conditions following anion exchange concentration, in the analysis of waters from the Solent and the English Channel⁴. These were found to contain lower amounts of mercury, from 0.014 to 0.021 μg /l., which are quite similar to that (0.03 μg /l.) found for a single sample from off Heligoland in the early work of Stock and Cucuel⁵.

Table 1 Mercury in Water Samples

Discovery station No.	Position	Depth (m)	Mercury (μg /l.)
7555	47° 22' N 7° 38' W (bottom at 3,927 m)	about 1,000 > 2,000*	0.006 0.020
7556	46° 35' N 8° 28' W (bottom at 4,743 m)	0 about 1,000 4,665	0.015 0.013 < 0.003
7560	47° 22' N 7° 37' W (bottom at 3,858 m)	3,506	0.015
7602	44° 47' N 9° 10' W	0	0.017
7604	40° 42' N 9° 31' W	0	0.018
7605	39° 32' N 9° 35' W	0	0.013

* There is uncertainty about the operation of the two sampling bottles which tripped at 2,000–2,200 m. Either one or both of the bottles may have sampled at that depth or at 3,700 m.

These data represent a situation, not unusual in analytical marine geochemistry, where interpretation is difficult not just because there are only a few results for widely separated areas but also because the differences may reflect analytical effects as well as real regional variations. In view of the increasing interest in the marine distribution of mercury, related to its local importance as a pollutant, more information is needed. The results are given here for analyses of ocean water samples from the north-eastern Atlantic, collected in late February and early March 1971. Surface samples (30 l.) were taken with a plastic bucket and subsurface samples (15 l.) with polypropylene water bottles. They were stored in polyethylene bottles after acidification to 0.1 N with purified hydrochloric acid and addition of ²⁰³Hg as an isotopic dilution tracer. Analysis was carried out by the above-mentioned procedure which has been outlined in more detail before⁴ and which recovers dissolved mercury present either in inorganic forms or as methylmercury. The samples were not filtered; they contained little particulate material and previous work⁵ has shown that this would contribute negligibly to the measured mercury.

The results are given in Table 1. The concentrations in the four surface samples were between 0.013 and 0.018 μg /l.; those in three deep water samples were closely similar but two other deep samples showed markedly lower concentrations.

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- ¹ Hosohara, K., Kozuma, H., Hawasaki, K., and Tsuruta, T., *J. Chem. Soc. Japan, Pure Chem. Sect.*, **82**, 1479 (1961).
- ² Kurland, T. L., Faro, S. N., and Siedler, H., *Wld Neurol.*, **1**, 370 (1960).
- ³ Hosohara, K., *J. Chem. Soc. Japan, Pure Chem. Sect.*, **82**, 1107 (1961).
- ⁴ Burton, J. D., and Leatherland, T. M., *Nature* (in the press).
- ⁵ Stock, A., and Cucuel, F., *Naturwissenschaften*, **22**, 390 (1934).

Accelerated Rates of Rainfall

RAINFALL intensity within clouds may be greatly increased by the production of satellite drops when raindrops collide, and this process could explain the extremely high rates of rainfall development reported in certain clouds^{1,2}. We have set out to test this possibility by laboratory experiments.

Two streams of uniformly sized drops, of radii R and r , were made to collide while falling through air with a relative velocity U . Photographs were taken of the collision events over a wide range of values of R , r , U and the collision parameter X which is the perpendicular distance from the centre of one drop to the trajectory of the centre of the other. In general it was found that for low velocity central collisions the drops coalesced permanently but that for higher velocity collisions at larger values of $X/R+r$ the drops temporarily coalesced but then rotated and separated, usually drawing out a thin filament of water, several drop diameters in length, which condensed to form a small number of satellite drops (Fig. 1). Calculations based on the assumption that the drops will separate if their rotational energy exceeds the surface energy required to form two drops of the original size predict a critical threshold for separation given by the equation

$$\frac{r\rho U^2}{\sigma} \left(\frac{X}{R+r} \right)^2 = 3.2\gamma(R/r)$$

where ρ is the density and σ the surface tension of water and $\gamma(R/r)$ is a function which varies from 1 for $R=r$ to 2.9 for $R=3r$. Excellent agreement with this equation was found experimentally. The average number of satellite drops produced when separation occurred was three, but it varied from one to about eight. This was found to be fairly insensitive to quite wide variations in U , R , r and $X/(R+r)$. The average radius of the satellites is seen from Fig. 2 to be about 80 μm , corresponding to a raindrop with terminal velocity of $\sim 0.6 \text{ ms}^{-1}$.

If raindrops falling through clouds suffer such collisions the rate of transformation of cloud water to rainwater will be

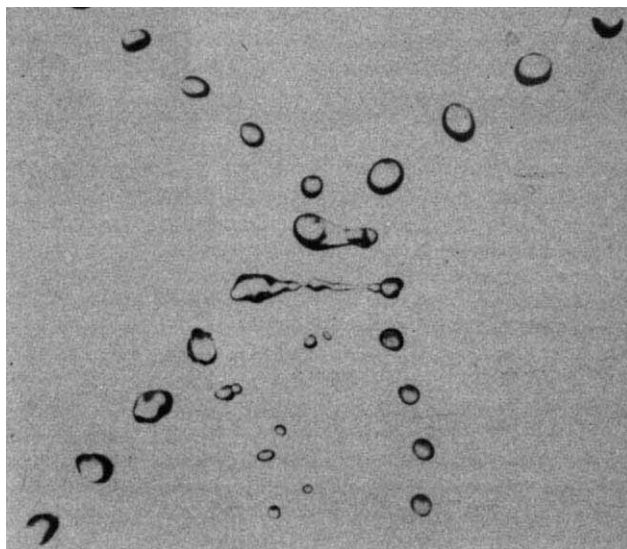


Fig. 1 Two water drops of radii 510 and 340 μm colliding in air and separating to form satellite drops.

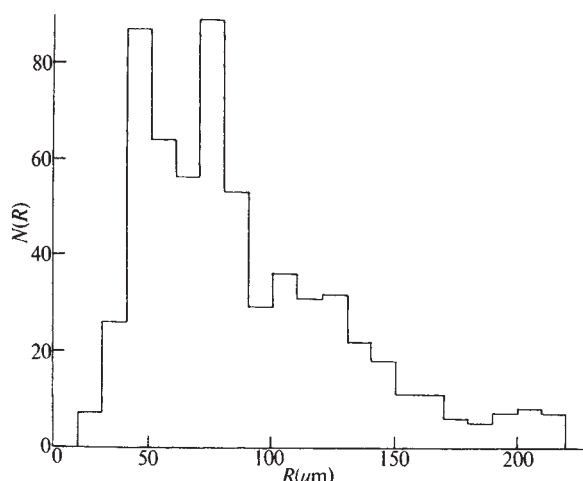


Fig. 2 Histogram of the number $N(R)$ of satellite drops of radius R produced by the collision and separation of falling water drops.

increased because the satellites will contribute to this sweepout process. The present work demonstrates that at terminal velocity satellites will be produced most readily by the glancing collisions of drops whose radius ratio R/r is between 2 and 3. Smaller values of R/r have relative velocities that are too small to produce appreciable separation, and for larger values the rotational energy generated by the collision is insufficient to cause separation.

Calculations have been made, on the basis of the Marshall-Palmer raindrop size distribution³, of the influence of satellite drop production on the rate of sweepout of cloud water. It was found that satellites are important in this transformation process, particularly in clouds of high liquid water content. The drops in the parent population must interact for several minutes in order to modify significantly the sweepout rate, but thereafter this secondary process becomes increasingly powerful and can become predominant after 10 or 15 min.

A more detailed assessment of the role of satellites in rainfall production is being made, but it seems possible that this process could explain the extremely rapid rates of rainfall development which have been observed in certain clouds by several workers^{1,2}.

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¹ Moore, C. B., and Vonnegut, B., *Physics of Precipitation*, Geophys. Monog. No. 5 (Amer. Geophys. Union, Washington, 1960).

² Muchnik, V. M., *Studies of Clouds, Precipitation and Thunderstorm Electricity*, 219 (AMS, Boston, 1965).

³ Marshall, J. S., and Palmer, W. M., *J. Meteorol.*, 5, 165 (1948).

Evaluation of Ice Nuclei Generator Systems

ARTIFICIAL ice nuclei, generated by combustion of acetone solutions of silver iodide¹, have been used in cloud seeding programmes since the early 1950s, with variable, and sometimes disappointing, results. It is apparent that the ice nuclei

disseminated in many field projects have been complexes, or intimate mixtures, of silver iodide and the alkali iodides, their hydrates and decomposition products, rather than silver iodide. Although the basic evidence has been available in the published literature, the possibility that such complexes could function as ice nuclei in a different manner than would uncomplexed silver iodide has been either not understood or largely ignored.

Silver iodide is insoluble in acetone; its complexes with sodium, potassium and ammonium iodides are, however, readily soluble. Useful solution occurs at a minimum, molar complexing ratio of two silver iodides to one alkali iodide²; the silver iodide-sodium iodide system has been most commonly used for ice nuclei generation.

On combustion, acetone solutions of silver iodide and either sodium or potassium iodide yield their inorganic ingredients essentially unchanged. Neither silver iodide nor the alkali iodides are destroyed by the transient high temperatures or by oxidative-reductive reactions encountered in a properly regulated generator flame. Evidence³ suggests strongly that silver iodide does not segregate from the alkali iodide during combustion. The solid products obtained must initially be intimate mixtures or chemical complexes of silver iodide and the alkali iodides in the same molar ratios as in the generator feed solutions.

Mixtures and complexes of silver iodide and alkali iodides are hygroscopic. Exposure to air of relative humidity < 40% leads to the formation of stable hydrated complexes such as $2 \text{AgI} \cdot \text{KI} \cdot 0.5 \text{H}_2\text{O}$ and $2 \text{AgI} \cdot \text{NaI} \cdot 3 \text{H}_2\text{O}$. Hydration at higher relative humidities leads to partial or complete solution, followed by eventual precipitation of silver iodide. The particle size of the precipitated silver iodide is variable and depends on the rates of hydration and dilution of the nucleus particle and the subsequent ageing of the precipitate.

When complex nuclei are dispersed in the atmosphere, exposure to moist air or cloud environments warmer than 0° C will result in the chemical and physical changes outlined above. The utility of these materials, now degraded as ice nuclei, is apparently manifested only at temperatures below -12° C to -14° C. A. Auer and D. Veal, of the University of Wyoming, find that the silver iodide-sodium iodide complex functions effectively as ice nuclei only at temperatures of -12° C and lower at Elk Mountain, Wyoming, when they are generated below, and introduced into, the cap cloud base. R. Steele of the Desert Research Institute, University of Nevada, reports that these complexes function as ice nuclei only at temperatures below -14° C if introduced into a dynamic cloud chamber at temperatures above 0° C and before expansion to produce the cloud. Laboratory experiments reflect the enhanced solubility of these nuclei; refreezing of melted ice crystals which had been generated by silver iodide-sodium iodide nuclei, occurs at temperatures below -12° C (ref. 4).

Uncomplexed, silver iodide nuclei exhibit none of the hydration-solubility-precipitation characteristics of complex nuclei. Barring ultimate solution in water or possible photo-degradation, silver iodide aerosols are relatively indifferent to atmospheric exposure and withstand limited passage through warm cloud environment. The silver iodide-ammonium iodide-acetone combustion process⁵ seems to be ideally suited for the generation of uncomplexed silver iodide aerosols. Silver iodide solutions in acetone at concentrations up to 25% by weight are easily obtained using the minimum molar complexing ratio of ammonium iodide ($2 \text{AgI} \cdot \text{NH}_4\text{I}$), providing that some water is included in the formulations.

Ammonium iodide is destroyed on combustion of its acetone solutions. X-ray diffraction studies show that the solid product obtained on combustion of silver iodide-ammonium iodide-water solutions is uncontaminated silver iodide. These results are independent of solution concentrations. The silver iodide is, as expected, predominantly the face centred cubic (γ) crystal modification⁶. Preliminary nucleation studies conducted

in the cloud chamber facility⁷ of the South Dakota School of Mines and Technology show that a 5% by weight silver iodide solution using ammonium iodide as the solubilizing agent will yield 2×10^9 ice crystals per g of AgI at -3.5°C and 10^{14} per g at -20°C . Under the same test conditions, an equivalent solution of silver iodide, using sodium iodide as a solubilizing agent, yields no nuclei active above -7°C and 2×10^{14} ice crystals per g of AgI at -20°C .

When the freezing process is to be invoked for successful cloud seeding, the freezing agents should be effective at the warmest possible temperatures, in order to take advantage of the higher concentrations of liquid and vapour water, to promote the maximum dynamic response, and to compete best with naturally occurring nuclei. Clearly, complexed nuclei, generated at warm cloud base or out of cloud, are not the optimum for these purposes.

If ice nuclei exhibit behaviour dependent on their history of environmental exposure and their chemical composition, then field experimentation using such nuclei may have yielded results culminating in confusion as to the true effects of cloud seeding. A re-evaluation of the effects of cloud seeding in Project Whitetop⁸, and in the Arizona cumulus project of Battan⁹, in light of the foregoing might help to clarify some statistical analyses in which the effectiveness of cloud seeding has been questioned¹⁰. Comparison of these programmes, which used complexed silver iodide aerosols, with others, such as the winter storm project in Santa Barbara, California¹¹, and the cold cumulus cloud seeding experiments in Oklahoma¹² where relatively pure silver iodide was used, should prove enlightening.

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- ¹ Vonnegut, B., *J. Colloid Sci.*, **5**, 37 (1950).
- ² Ishii, S., *Rep. Osaka Imp. Ind. Res. Inst. Japan*, **11**, 25 (1931).
- ³ Mossop, S. C., and Tuck-Lee, C., *J. Appl. Meteor.*, **7**, 234 (1968).
- ⁴ Vali, G., *J. Rech. Atmos.*, **3**, 175 (1968).
- ⁵ Vonnegut, B., *Chem. Rev.*, **44**, 277 (1949).
- ⁶ Burely, G., and McMurdie, H. F., *NBS Rep.*, No. 6557 (Government Printing Office, Washington, 1959).
- ⁷ Donnan, J. A., Blair, D. N., Finnegan, W. G., and St-Amand, P., *J. Weather Modification*, **2**, 155 (1970).
- ⁸ Braham, R. R., *Final Report of Project Whitetop*, Parts I and II (Univ of Chicago, 1966).
- ⁹ Battan, L. J., and Kassander, A. R., Scientific Report No. 18 (Institute of Atmospheric Physics, Univ. of Arizona, 1962).
- ¹⁰ Neyman, J., Scott, E. L., and Smith, J. A., *Science*, **163**, 1445 (1969).
- ¹¹ Elliott, R. D., and St-Amand, P., *J. Appl. Meteor.* (in the press).
- ¹² Sutherland, J. L., Cooper, L. W., and Booker, D. R., *J. Weather Modification*, **3**, 115 (1971).
- ¹³ Booker, D. R., Sutherland, J., Cooper, L., and St-Amand, P., *J. Appl. Meteor.* (in the press).

BIOLOGICAL SCIENCES

Dimethylbenzanthracene Binding to Epidermal Chalone

THE substance called epidermal chalone contained in normal mammalian epidermis is known to inhibit mitosis in a tissue specific manner¹⁻³. The chalone is heat labile and nondialysable; it is precipitated by ethanol and can be preserved in a lyophilized form⁴. It appears⁵ to be a glycoprotein of molecular weight between 25,000 and 30,000. Aqueous extracts and a partially purified ethanol fraction of the skin of man, mouse,

cod and pig inhibit mitosis *in vitro* and *in vivo*^{4,6,7}. Similar extracts from other tissues do not affect mitosis in the epidermis⁸⁻¹².

Bullough^{4,13,14} considers that the epidermal chalone maintains the balance between cell production and cell loss in the epidermis. Disruption of this normal balance by a lack of mitotic control is a distinctive feature of neoplasia, and so it is possible that the chalone plays some part in carcinogenesis. Indeed, the deletion of tissue specific growth regulating proteins by carcinogens was considered¹⁵ early in the 1940s, and the binding of carcinogens (but not of noncarcinogens) to a specific soluble protein fraction of mouse skin and rat liver has been reported¹⁶⁻²⁰.

We have therefore investigated the binding of 9,10-dimethyl-1,2-benzanthracene-9-¹⁴C (DMBA-¹⁴C) to epidermal chalone. As a control we used anthracene-9-¹⁴C, a known noncarcinogen. Bullough²¹ claims that a reduction in the concentration of chalone in a cell results in circumstances favourable to cell division. A carcinogen such as DMBA might bind the epidermal chalone, thus decreasing the intracellular chalone concentration and resulting in cell division.

As a preliminary experiment, chalone was prepared from pork rind as described by Bolding and Laurence⁵. The presence of an inhibitor to mitotic activity in the rind water extracts, as well as an alcohol fraction, was shown by incubating fragments (3 × 5 mm) of mouse ear skin in the presence and absence of the tested material and counting the mitosis arrested by added colchicine as described by Bullough *et al.*^{1,22}. Addition of 15 mg of lyophilized water extract per 4 ml. of the incubation medium inhibited mitosis by 48%, and 10 mg of a partially purified lyophilized alcohol fraction precipitated at 72% caused a 50% decrease in the number of mitoses arrested.

The binding of the carcinogen DMBA to epidermal chalone was then investigated. The backs of male mice (Swiss Webster ICR-Blue Spruce Farm, New York) 10-12 weeks old were clipped. After 24 h, 9,10-dimethyl-1,2-benzanthracene-9-¹⁴C (DMBA-9-¹⁴C) in acetone (70-100 µg per mouse, 50 µCi/1.96 mg) or anthracene-¹⁴C (Amersham/Searle) in acetone (55-70 µg per mouse, 180 µCi/1 mg) was applied to the back with a special micropipette. After 6, 24 or 48 h the animals were killed either by ether or by cervical dislocation. The backs were sponged with cotton and soaked in acetone several times to remove any free hydrocarbon adhering to the skin, which, after the removal of adipose tissue and blood vessels by scrap-

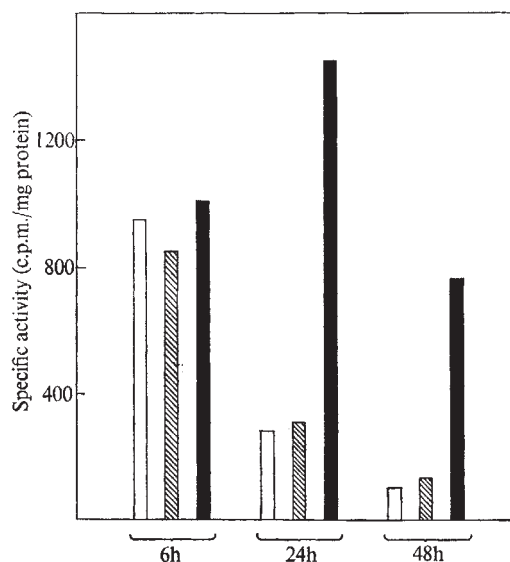


Fig. 1 The binding of DMBA-¹⁴C to various fractions of skin precipitated at 55% (white columns); 55-70% (hatched columns); and 70-80% (black columns) ethanol respectively. Skin was extracted after application of carcinogen for 6, 24 and 48 h.

Table 1 Binding of DMBA-¹⁴C and Anthracene-¹⁴C to the Different Alcohol Fractions and to the Water-soluble Extracts of Mouse Skin at Various Times after Topical Application

Compound tested	Time after application (h)	No. of mice used	Specific activity of alcohol fractions (c.p.m./mg of protein)		
			55%	55-70%	70-80%
DMBA	6	5	950	851	1,076
	24	5	295*	318*	1,444*
	48	5	112	133	768
Anthracene	6	5	11	17	0
	24	5	—	32	18
	48	5	0	7	0

* Results obtained by repeated experiments at the time indicated.

Table 2 Radioactivity in the Protein Bound Fraction precipitated at 70-80% ETOH Before and After Pronase Digestion, followed by Extraction and Thin-layer Chromatography

Treatment	Total c.p.m.	
	in water	in benzene
Before hydrolysis	2,941	
Pronase hydrolysate—dried and extracted	2,427	8
Thin-layer chromatography of hydrolysate. Origin eluted	457*	0
Area corresponding to marker DMBA	0	0

* Approximately 800 c.p.m. of hydrolysate were spotted on thin layers.

In all 457 c.p.m. were eluted from origin. None of the 350 c.p.m. lost were recovered anywhere else on plate.

ing, was frozen in liquid air²³. The epidermis from each group was ground in liquid nitrogen⁷ and extracted with saline. The mixture was then extracted and fractionated with pre-cooled 96% ethanol to final concentrations of 55, 70 and 80% (w/v) respectively, corrected for contraction according to Boldingh *et al.*⁵. The specific activity of each fraction, expressed as c.p.m. per mg of protein, was determined. Protein concentration was estimated by the method of Lowry *et al.*²⁴, and radioactivity determined by counting a sample of 0.1 ml. in 10 ml. of a scintillator solution in a 'Packard Tri-Carb' spectrometer. The specific activities of each of the alcohol-precipitated fractions of the epidermis are shown in Table 1. The binding of anthracene was clearly negligible in all fractions and the binding of DMBA was maximal in the alcohol fraction precipitated between 70 and 80% (Fig. 1). Maximum binding to this fraction occurred 24 h after topical application of the carcinogen. These results were confirmed several times. To test the strength of binding, the DMBA-¹⁴C bound fraction (1-2 mg) was incubated with the enzyme pronase (Calbiochem, 10-20 µg mg⁻¹ protein) at pH 7.5-8 at room temperature for 2 days in aseptic conditions. In other experiments, the DMBA-¹⁴C bound-fraction was hydrolysed with 5.6 M HCl in nitrogen at 110° C for 2 days, and in each case the hydrolysate (pronase or acid) was extracted with benzene or acetone. A portion of the organic solvent extract was counted for radioactivity and another chromatographed on commercial pre-coated plates of silica gel, using a mixture of n-hexane, O-dichlorobenzene and pyridine (10 : 1 : 0.5)²⁶, or CCl₄ (Table 2). With this method we were able to detect 0.3 µg of free DMBA. Very low counts were detected (6-10 c.p.m. above background) in the organic solvent extract, most of the counts remaining in the water soluble part of the hydrolysate. Furthermore, the radioactivity of the hydrolysate did not migrate on the thin-layer plate indicating that little, if any, was extracted by the organic solvent.

The 70-80% alcohol precipitate from the water-soluble extract of mouse skin from control, anthracene-¹⁴C and

DMBA-¹⁴C-treated animals was fractionated on 15% polyacrylamide gels. Acrylamide disc electrophoresis by the method of Panyim and Chalkley²⁷, based on the methods of Reisfeld *et al.*²⁷⁻²⁹, gave the best separation of several methods tried. Duplicate samples were also run. One was stained with 'Amido Black-10' (0.01% in 7.5% acetic acid) and the other cut into 2 mm slices to determine the radioactivity along the gel³⁰.

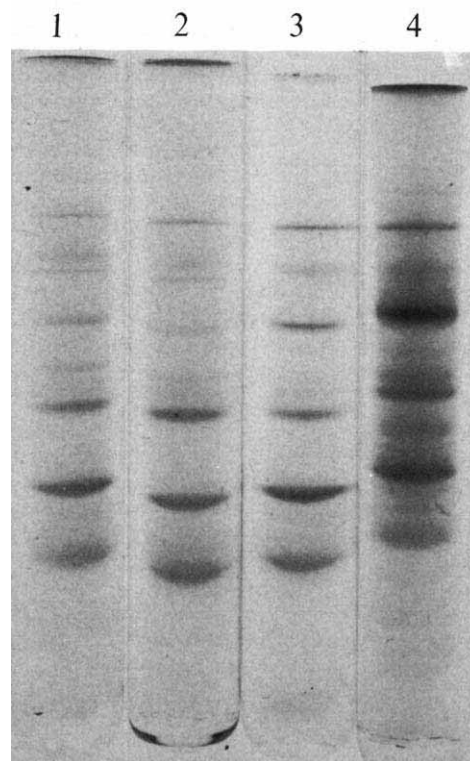


Fig. 2 Polyacrylamide gel electrophoresis of chalone fraction extracted 24 h after DMBA treatment and precipitated between 70-80% ethanol. 1, Control; 2, treated with anthracene-¹⁴C; 3, treated with DMBA; the gel was sliced lengthwise and half was used for radioactive counting and presented in Fig. 3; 4, treated with DMBA-¹⁴C, not sliced. Bands not found on control gels are marked by arrowheads.

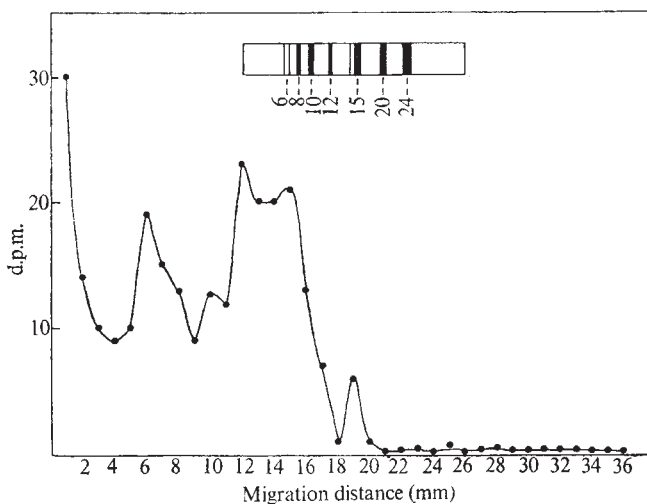


Fig. 3 The distribution of radioactivity along polyacrylamide, which was sliced lengthwise, and half stained to locate the bands. The stained gel is represented diagrammatically at the top of the figure showing the positions of the major bands. The unstained half was sliced into 2 mm pieces and the radioactivity measured.

The gel pattern presented in Fig. 2 shows that material from DMBA-treated animals has two light bands, which are not present in control or anthracene treated skin. The distribution of radioactivity is shown in Fig. 3. The peaks correspond to protein bands at positions 6, 12, 15, 20 and 24 of the gel. Most of the counts were associated with protein components migrating in the upper half of the gel.

The binding of carcinogens to water-soluble proteins has been suggested^{16,19} to be important in carcinogenesis. The specificity and cellular function of the protein binding have not yet been identified. The hypothesis that chalcones control mitotic homeostasis^{5,8,9} suggested that the carcinogen might bind to these growth regulating proteins.

We found that after mouse skin was painted with DMBA-¹⁴C, the highest specific activity (c.p.m. per mg of protein) was encountered in the fraction of the water-soluble protein precipitated with between 70 and 80% alcohol. This fraction has been reported² to contain more than 80% of the epidermal chalone. The binding to the 70–80% fraction seems to be covalent, because no labelled material was released after pronase digestion or acid hydrolysis of the bound fraction, followed by extraction with organic solvents and thin-layer chromatography. Covalent binding has been reported to occur between carcinogens and the soluble proteins of mouse skin^{5,23,31}.

The 70–80% fraction is very active in inhibiting the mitosis *in vitro* and *in vivo*⁵, and so it is possible that the protein to which the carcinogen binds is the chalone and, if so, this action may be of considerable importance in carcinogenesis. The 70–80% alcohol fraction is not, however, a pure protein, as shown by gel electrophoresis, which leaves the identity of the protein bound in doubt. Further purification of the alcohol fraction is difficult because of the very small quantity of chalone obtained in the final step⁵. Only if such a purified chalone is readily available will testing of the present hypothesis, that the effect of the carcinogen is to inactivate intracellular chalcones, thereby allowing mitosis to go unchecked, be possible.

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- ¹ Bullough, W. S., and Laurence, E. B., *Exp. Cell Res.*, **24**, 289 (1961).
- ² Bullough, W. S., Hewett, C. L., and Laurence, E. B., *Exp. Cell Res.*, **36**, 192 (1961).
- ³ Iversen, O. H., *Ciba Foundation Symp. Homeostatic Regulators* (edit. by Wolstenholme, G. E. W., and Knight, J.), 29 (Churchill, London, 1969).
- ⁴ Bullough, W. S., and Laurence, E. B., in *Adv. Biol. Skin*, 7 (edit. by Montana, W., and Dobson, R. L.) (Pergamon, New York, 1965).
- ⁵ Bolding, W. H., and Laurence, E. B., *Europ. J. Biochem.*, **5**, 191 (1968).
- ⁶ Bullough, W. S., Laurence, E. B., Iversen, O. H., and Elgjo, K., *Nature*, **214** (1967).
- ⁷ Iversen, O. H., Aandahl, E., and Elgjo, K., *Acta Pathol. Microbiol. Scand.*, **64**, 506 (1965).
- ⁸ Rytoymaa, T., and Kiviniemi, K., *Cell Tissue Kinetics*, **1**, 329 (1968).
- ⁹ Saetren, H. A., *Exp. Cell Res.*, **11**, 229 (1956).
- ¹⁰ Simnett, J. D., and Chopra, D. P., *Nature*, **222**, 1189 (1969).
- ¹¹ Voaden, M. J., and Leeson, S. J., *Exp. Eye Res.*, **9**, 57 (1970).
- ¹² Volm, M., Kinzel, V., Mohr, U., and Suss, R., *Experientia*, **25**, 68 (1969).

- ¹³ Bullough, W. S., *The Evolution of Differentiation* (Academic Press, London, 1967).
- ¹⁴ Bullough, W. S., and Laurence, E. B., *Europ. J. Cancer*, **4** (1968).
- ¹⁵ Schmidt, O., *Naturwissenschaften*, **29**, 146 (1941).
- ¹⁶ Heidelberger, C., *J. Cell Comp. Physiol.*, **64**, Suppl. 1, 129 (1964).
- ¹⁷ Miller, E. C., *Cancer Res.*, **11**, 100 (1951).
- ¹⁸ Miller, J. A., and Miller, E. C., *Adv. Cancer Res.*, **1**, 339 (1953).
- ¹⁹ Miller, E. C., and Miller, J. A., *Cancer Res.*, **12**, 547 (1952).
- ²⁰ Sorof, S., Young, E. M., and Fetterman, P. L., *Exp. Cell Res.*, **20**, 253 (1960).
- ²¹ Bullough, W. S., *Biol. Rev.*, **37**, 307 (1962).
- ²² Bullough, W. S., and Laurence, E. B., *Proc. Roy. Soc., B*, **151**, 517 (1960).
- ²³ Wiest, W. G., and Heidelberger, C., *Cancer Res.*, **13**, 246 (1953).
- ²⁴ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
- ²⁵ Patterson, M. S., and Greene, R. C., *Anal. Chem.*, **37**, 854 (1965).
- ²⁶ Matsushita, H., Suzuki, Y., and Shakabe, H., *Bull. Chem. Soc., Japan*, **36**, 1371 (1963).
- ²⁷ Panyim, S., and Chalkley, R., *Arch. Biochem. Biophys.*, **130**, 337 (1969).
- ²⁸ Reisfeld, R. A., Lewis, U. J., and Williams, D. E., *Nature*, **195**, 281 (1962).
- ²⁹ Clarke, J. T., *Ann. NY Acad. Sci.*, **121**, 428 (1964).
- ³⁰ Peter, V., Thishler, and Epstein, C. J., *Anal. Biochem.*, **22**, 89 (1968).
- ³¹ Abell, C. W., and Heidelberger, C., *Cancer Res.*, **22**, 931 (1962).
- ³² Mohr, U., Althoff, J., Kinzel, V., Suss, R., and Volm, M., *Nature*, **220**, 138 (1968).

Formation of N-Nitrosopiperidine from Piperidine and Sodium Nitrite in the Stomach and the Isolated Intestinal Loop of the Rat

SINCE the first report of hepatic cancer in rats induced by nitrosodimethylamine¹, many nitrosamines have been shown to be carcinogenic in a wide range of organs of a wide variety of species^{1–4}. Following the suggestion that dietary nitrite and secondary amines might interact in the human stomach to form nitrosamines⁵, such synthesis has been demonstrated *in vitro* with animal and human gastric juice^{6,7}, and *in vivo* in laboratory animals⁷ and man⁸; nitrosamine synthesis was pH-dependent^{7–10}. Nitrosamines can also be synthesized *in vitro* at near neutral pH values in the presence of enteric bacteria¹¹; yields of nitrosamines are also determined by the basicity of the secondary amine. Presumptive evidence for *in vivo* synthesis of nitrosamines has been demonstrated by the induction of tumours in rats following chronic feeding of nitrite and secondary amines¹¹. We report here the synthesis of nitrosopiperidine in rats from nitrite and piperidine in gastric contents *in vitro*, and in the stomach and in the small intestine *in vivo*.

Male Sprague-Dawley rats (Charles River Laboratories) weighing 150–300 g were fed Purina chow and water *ad libitum* before the day of test. Each rat was anaesthetized with sodium pentobarbital, the abdominal wall incised and the small intestine pulled out and isolated between ligatures at the gastro-duodenal and ileo-caecal junctions. A polyethylene tube (Intramedic, PE No. 50) was inserted into the upper end of the isolated intestine and a third ligature tightened around it. The intestinal loop was then isolated by section above the ileo-caecal ligature, and flushed with saline through the polyethylene tubing. Finally, the open end of the washed loop was ligated. Solutions of various concentrations of sodium nitrite and of piperidine hydrochloride (5 ml.) (Table 1) were introduced into the intestinal loop through the tubing. The loop, with its vasculature intact, was then returned to the abdomen of the anaesthetized rat for 40 min; the abdominal incision was covered by a warmed saline pad. Intestinal contents were subsequently washed out with saline and collected. The pH of the intestinal contents was measured with a Beckman meter before and after incubation. *In vivo* experiments were conducted concurrently with intestinal loop

Table 1 *In vivo* Formation of Nitrosopiperidine from Piperidine Hydrochloride and Sodium Nitrite in the Isolated Intestinal Loop and the Isolated Stomach of the Rat

Replicate	Reactant concentration (mg)		Total volume of reactants (ml.)	Nitroso- piperidine yield * (µg)	pH
	Piperidine HCl	Sodium nitrite			
Small intestine					
1	125	25	10	0	6.5
2	125	25	10	0	6.5
3	125	25	10	0	6.5
4	125	25	10	0	6.6
5	625	25	10	25	6.5
6	625	25	10	21	6.6
7	625	25	10	5	6.6
8	1,250	25	10	159 †	6.5
9	1,250	25	10	157	6.5
Stomach					
1	625	25	10	12	3.0
2	625	25	10	24	4.0
3	312.5	12.5	5	11	5.4
4	250	10	4	22	3.6
5	500	10	4	19	4.2
6	500	10	4	46	3.6
7	500	10	4	23	4.2

* Estimated by GLC.

† Confirmed by mass spectroscopy.

studies in some rats. The stomach was isolated between ligatures at the oesophago-gastric and gastro-duodenal junctions. A polyethylene tube was inserted through the latter and a third ligature tightened around it. Solutions of various concentrations of nitrite and piperidine (Table 1) were then introduced through the polyethylene tube. The stomach, with its intact vasculature, was then returned to the abdomen of the rat for 40 min. The stomach contents were subsequently washed out with saline and collected. Intestinal washings and stomach contents, excluding coarse food particles, were incubated aerobically with constant shaking on a mechanical shaker, for 30 min with nitrite and piperidine at 37° C (Table 2).

Nitrosopiperidine was extracted by described methods earlier⁷. The intestinal or stomach contents along with the washings, in volumes of 60–80 ml., were filtered through Whatman No. 1 paper to remove coarse particles. The filtrate was passed through anion ('Amberlite IRA-400', chloride form) and cation ('Rexyn 101', hydrogen form) exchange columns; columns were then washed with distilled water. The combined washings and effluent were extracted twice with 150–200 ml. aliquots of methylene chloride; emulsions forming during solvent extraction were broken by centrifugation. The extract was dried over anhydrous sodium sulphate, and the solvent was distilled off at 37° C until 15–20 ml. of the extract remained. This was transferred to a centrifuge tube and evaporated to dryness under nitrogen. The residue was dissolved in 0.1–0.2 ml. methylene chloride. An aliquot of this solution was used for the estimation of nitrosopiperidine by gas liquid chromatography and for its identification by thin-layer chromatography (TLC)¹². An F and M 500 gas chromatograph equipped with 1609 hydrogen flame ionization detector and 1 mV recorder was used. Gas liquid chromatography (GLC) conditions were: 6% coating of 'Reoplax 400' on 60–80 mesh chromosorb W, in stainless steel column 5' × 1/8" inner diameter; helium flow rate at delivery pressure of 26–30 pounds/inch² and rotameter setting at 6.2–6.5, column temperature 100°–115° C, range setting at 100 and attenuation 2; 1–2 µl. samples were injected into the GLC column. The limit of detection of nitrosopiperidine in 0.1–0.2 ml. of total extract was 1 µg. The mean zero time recoveries of 5–10 mg of nitrosopiperidine added to saline, and to intestinal contents *in vitro*, were 23% and 22%, respectively. The mean recoveries

of 10 mg nitrosopiperidine at zero time and after 40 min in the small intestine were 10% and 3%, respectively.

Nitrosopiperidine was synthesized *in vivo* in the small intestine of rats from nitrite and piperidine (Table 1). With a constant nitrite concentration, the yield of nitrosopiperidine was dependent on the piperidine concentration, and varied from 5 to 159 µg. The identity of nitrosopiperidine was established by a combination of techniques. After TLC separation the spot with the same R_F value as standard nitrosopiperidine was extracted and injected into a GLC column. A single peak with the same retention time as authentic nitrosopiperidine was obtained. The presence of nitrosopiperidine in an extract was confirmed by mass spectrometry, using an AEI-MS-9 instrument. A peak at m/e 114 was obtained; accurate mass measurement showed it to be 114.0794; $C_5H_{10}N_2O$ (nitrosopiperidine) = 114.0793. Nitrosopiperidine was also synthesized *in vivo* in the rat stomach from piperidine and nitrite in concentration ratios of 25/1 and 50/1 (Table 1); yields of nitrosopiperidine ranged from 11–40 µg. Higher yields of nitrosopiperidine, ranging from 327–785 µg, were, however, found *in vitro* in gastric juice (Table 2). Nitrosopiperidine was not, however, synthesized *in vitro* from its precursors in small intestinal washings (Table 2).

Table 2 *In vitro* Formation of Nitrosopiperidine from Piperidine Hydrochloride and Sodium Nitrite in Small Intestine Washing and in Gastric Juice of the Rat

Replicate	Reactant concentration (mg)		Total volume of reactants (ml.)	Nitroso-piperidine yield (µg)	pH
	Piperidine HCl	Sodium nitrite			
Small intestine washing					
1	1,250	25	10	0	6.5
2	1,250	25	10	0	6.6
3	1,250	25	10	0	6.7
Gastric juice					
1	1,250	25	10	327	4.0
2	1,250	25	10	346	4.0
3	1,250	25	10	785	4.0

The yields of nitrosopiperidine reported here are probably underestimates in view of the low recoveries. Furthermore, no effort was made to correct for nitrosopiperidine absorbed during 40 min *in vivo*. This may also account for the relatively lower yields of nitrosopiperidine from the stomach than from gastric contents *in vitro*. The failure to detect nitrosopiperidine in intestinal contents *in vitro* may reflect the unfavourable condition of incubation. Nitrosopiperidine is a potent carcinogen producing tumours of the oesophagus in rats, as well as at other sites, after oral, intravenous or subcutaneous administration³. The oral carcinogenic dose of nitrosopiperidine in rats is 5 mg/kg for 9–12 months, equivalent to a total lifetime dose of 2,000 mg/kg³; but no reports of dose response studies are available on the carcinogenicity of nitrosopiperidine. Our studies show the synthesis of nitrosopiperidine in the rat from nitrite and piperidine in gastric juice *in vitro* and in the stomach and small intestine *in vivo*. The *in vivo* synthesis of nitrosopiperidine from a single dose of nitrite and piperidine, in yields ranging from 5–159 µg and 11–40 µg in small intestine and stomach, respectively, is noteworthy especially because these yields are underestimates as a result of low recovery.

Piperidine may be ingested by man through some foods and spices. It can also be synthesized from the dietary lysine by intestinal bacteria¹³. The average amounts of piperidine in urine of normal healthy subjects were found to be 1,236 µg/day; tetracycline administration decreases urinary piperidine¹⁴.

This demonstration of synthesis of a nitrosamine *in vivo* in the small intestine is unique and consistent with previous reports on the role of bacteria in biosynthesis of nitrosamines *in vitro*⁹. The role of acid pH conditions in nitrosamine biosynthesis is thus probably less critical and less limiting than has hitherto appeared. Our findings thus confirm and extend previous reports on the synthesis of other nitrosamines from their nitrite and secondary amine precursors, both *in vitro*^{6-10,15} and *in vivo*^{7,8}.

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- ¹ Magee, P. N., and Barnes, J. M., *Brit. J. Cancer*, **10**, 114 (1956).
- ² Magee, P. N., and Barnes, J. M., *Adv. Cancer Res.*, **10**, 163 (1967).
- ³ Druckrey, H., Preussman, R., Ivankovics, S., and Schmahl, D., *Z. Krebsforsch.*, **69**, 103 (1967).
- ⁴ Schmahl, D., and Osswald, H., *Experientia*, **23**, 497 (1967).
- ⁵ Druckrey, H., Steinhoff, D., Beuthner, H., Schneider, H. V., and Klarner, P., *Arzneimitt-Forsch.*, **13**, 320 (1963).
- ⁶ Sander, V. J., *Arch. Hyg. Bakt.*, **151**, 22 (1967).
- ⁷ Sen, N. P., Smith, D. C., and Schwinghamer, L., *Food Cosmet. Toxicol.*, **7**, 301 (1969).
- ⁸ Sander, V. J., and Seif, F., *Arzneimitt-Forsch.*, **19**, 1091 (1969).
- ⁹ Sander, V. J., *Z. Physiol. Chem.*, **349**, 429 (1968).
- ¹⁰ Mirvish, S. S., *J. Nat. Cancer Inst.*, **44**, 633 (1970).
- ¹¹ Sander, V. J., Schweinsberg, F., and Menz, H. P., *Z. Physiol. Chem.*, **349**, 1691 (1968).
- ¹² Sen, N. P., Smith, D. C., Schwinghamer, L., and Marleau, J. J., *J. Assoc. Anal. Chem.*, **52**, 47 (1969).
- ¹³ Blau, K., *Biochem. J.*, **80**, 193 (1961).
- ¹⁴ Kasé, Y., Kataoka, M., and Miyota, T., *Jap. J. Pharmacol.*, **19**, 354 (1969).
- ¹⁵ Lijinsky, W., and Epstein, S. S., *Nature*, **225**, 21 (1970).

Dechlorination of DDT in Solution by Ionizing Radiation

THERE have been several studies of the effects of ultraviolet light on DDT¹⁻⁵ but little is known of the chemical effects of ionizing radiation on the pesticides. In the light of the availability of long lived radioisotopes such as ⁶⁰Co and ¹³⁷Cs, by-products of the nuclear power industry, and of the current concern about pollution of lakes and rivers by persistent pesticides, we undertook to study the feasibility of dechlorinating DDT solutions by exposure to ⁶⁰Co γ -rays. Because of the low efficiency with which ionizing radiation absorbed by materials is converted into chemical energy⁶, however, it is evident that to be of economic importance, any radiation-initiated process must involve a chemical chain mechanism.

In contrast to its high solubility in lipids and other organic materials, DDT is almost insoluble in pure water⁷. The bulk of DDT carried in contaminated water is probably in an organic environment, dissolved in suspended liquid fats, in soap and in detergent micelles, or adsorbed on microparticulates. We therefore turned first to the study of radiation dechlorination of DDT in organic solvent. 2-Propanol was chosen because it had proved to be a suitable solvent in previous work on the mechanism of chain dechlorination of carbon

Table 1 Dependence of Yield on Radiation Time in the γ Radiolysis of De-aerated Solutions of DDT in 2-Propanol*

Dose rate† (10^{17} eV ml. ⁻¹ min. ⁻¹)	Time (min)	HCl (mM)	Acetone (mM)	DDD (mM)	DDT consumed (mM)
13.6	5	3.0	2.9	—	—
13.6	10	5.8	5.0	4.6	5.7
13.6	20	12	10	5.4	11
13.6	30	18	18	9.9	15
0.49	30	2.0	—	—	—
0.49	45	3.5	—	—	—
0.49	90	5.8	5.2	5.5	6.5
0.49	150	9.7	9.2	9.2	9.2
0.49	270	16	15	14	15

* Initial DDT concentration, 20 mM.

† Measured by Fricke dosimetry, with correction for the lower electron density of 2-propanol.

tetrachloride⁸, which may be considered a structural model for DDT.

Solutions of DDT in 'Pyrex' tubes were irradiated in anaerobic conditions. Products identified were hydrochloric acid, acetone and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD). A comparison of the data for the lowest and highest radiation dose rates used (Table 1) shows that at the low dose rate these products conform to the stoichiometry



while at the high dose rate the DDD yield is consistently smaller than the DDT consumption. Inspection of the results for the three other dose rates used (7.50 , 4.58 and 2.14×10^{17} eV ml.⁻¹ min.⁻¹) shows that while the lowest dose rate data conform with the above stoichiometry, there is a progressive disparity between DDD formation and DDT consumption in the two sets of higher dose rate data. At all dose rates HCl, acetone and -DDT yields were essentially equal, with a linear dependence on dose up to approximately 100% consumption of DDT (Fig. 1). Curves I-V represent dechlorination (*G*) values (molecules per 100 eV) of 25, 29, 34, 43 and 81, respectively.

To determine whether dechlorination of DDT involves free radical intermediates, radiolysis was carried out in the presence of small concentrations ($\sim 10^{-2}$ M) of free radical scavengers such as O₂, I₂, DPPH, nitrobenzene and phenyl disulphide. In all cases dechlorination yields were reduced significantly. If it is assumed that in irradiated 2-propanol the primary yield of radicals which are amenable for reaction with a solute is

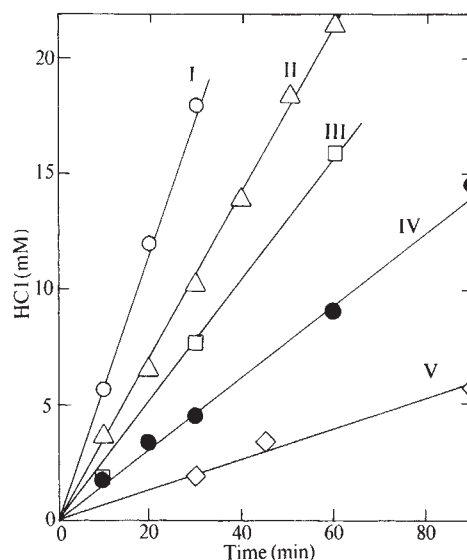


Fig. 1 Effect of dose rate on the dechlorination of DDT in 2-propanol. Dose rates for curves I, II, III, IV and V are respectively 1.36 , 0.75 , 0.46 , 0.21 and 0.049×10^{18} eV ml.⁻¹ min.⁻¹.

6.7 radicals/100 eV⁹, then at all dose rates used in this study, *G* (dechlorination) values in the absence of added scavenger exceed significantly the radiolytic yield of radicals. This would indicate a chain process. If it is also assumed that all these radicals are successful in initiating the chain, then at the lowest dose rate (where maximum *G* values occur) the chain length must be 12. If, on the other hand, initiation is less than 100% efficient, this estimate of the chain length is only a lower limit.

The preceding data for DDT are consistent with the same free-radical chain mechanism postulated for the carbon tetrachloride-2-propanol system⁸. The dependence of yield on radiation intensity indicates that chain termination is second order in chain carriers, while the rather short chain length suggests that the rate constant for the rate-determining propagation step is an order of magnitude lower than that for the carbon tetrachloride reaction.

This is the first reported observation of a dehalogenation of a persistent pesticide which involves a chain mechanism. Because of the apparent low rate constant for one of the propagation steps in the chain, it would seem that the small amounts of secondary alcohols present in water contaminated with DDT could not sustain long chains in competition with chain-terminating impurities such as dissolved O₂ which are present in natural conditions. Thus, while the reaction we have described may not be an economic way of treating water contaminated with DDT, it certainly offers a basis for further research.

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- ¹ Linquist, R. A., Jones, H. A., and Madden, A. H., *J. Econ. Entomol.*, **39**, 55 (1946).
- ² Wichmann, J. J., Patterson, W. I., Clifford, P. A., Klein, A. K., and Claborn, H. V., *Amer. Assoc. Off. Agric. Chem.*, **29**, 222 (1946).
- ³ Mosier, A. R., Guenzi, W. G., and Miller, L. L., *Science*, **164**, 1083 (1969).
- ⁴ Miller, L. L., and Narang, R. S., *Science*, **169**, 368 (1970).
- ⁵ Plimmer, J. R., Klingbiel, V. I., and Hummer, B. E., *Science*, **167**, 67 (1970).
- ⁶ Hart, E. J., *J. Phys. Chem.*, **56**, 595 (1952).
- ⁷ Bowman, M. C., Acree, F., and Corbett, M. K., *J. Agric. Food Chem.*, **8**, 406 (1960).
- ⁸ Radlowski, C., and Sherman, W. V., *J. Phys. Chem.*, **74**, 3043 (1970).
- ⁹ Adams, G. E., Baxendale, J. H., and Sedgwick, R. D., *J. Phys. Chem.*, **63**, 854 (1959).
- ¹⁰ Sherman, W. V., in *Advances in Free-Radical Chemistry*, **3** (Academic Press, New York, 1969).

Biphasic Occurrence of IgM-forming Cells in the Intestine after Oral Immunization

THE lamina propria has its own lymphatic organ with immunocompetent cells. The secretions of the mucous membranes contain, besides IgG and IgM, predominantly immunoglobulin IgA¹, which seems to be produced in the lymphatic part of the lamina propria and secreted into the intestinal lumen². Here the epithelial cells may play a special role by

producing the so-called T piece that links 7S IgA to an 11S unit³.

Here we report IgM-producing cells which are demonstrable in the lamina propria by the direct Jerne technique and which occur biphasically after oral immunization. Five animals from each group were examined from the third to the twenty-seventh day after immunization (Table 1). The animals were killed on the days of examination, and the spleens and 15 cm segments of intestine were immediately removed aorally from the pylorus. The Peyer's patches were removed and the residual intestine was used for preparation. Cell suspensions in Eagle's medium were prepared from spleen and intestinal tissue in Potter-Elvehjem homogenizers using slow rotation. Fibres and foreign matter were eliminated by centrifugation at 500 r.p.m. The supernatant contained cells with minimal contamination. To tubes containing 5 ml. of intestinal cell suspension we added 2,000 KIU (kallikrein inactivating units) of trasylol (a protease inhibiting polypeptide), 0.1 g/ml. of canamycin sulphate and 50 IU of hyaluronidase. After the cell suspensions had been washed three times with Eagle's medium the number of cells in each cubic millimetre were counted. Smears of the intestinal cell suspensions showed that 90% of the cells were lymphocytes or plasma cells at different stages of maturation.

According to the agar-plaque technique^{5,6}, 0.1 ml. of cell suspension and 0.2 ml. of a 1% SRBC suspension (in 1 ml. of 1.4% 'Agarose' with 1 ml. of doubly concentrated Eagle's medium and 0.1 ml. of modified barbital buffer⁴) were added and the mixture was poured on to an agar-base layer at 45° C. After 1 h of incubation at 37° C the preparation was layered by 1 ml. of guinea-pig complement (diluted 1:10). After incubation for a further 30 min the plaque number per 10⁶ cells was estimated.

Table 1 Number of Plaque Forming Cells after Oral and Intravenous Immunization

Days after termination of immunization	Intravenous immunization		Oral immunization	
	Spleen	Intestine p.f.c./10 ⁶ cells	Spleen	Intestine
3	166	0	2	2
4	1,455	7	2	2
5	388	5	4	4
6	34	0	7	4
7	38	39	43	45
10	47	13	22	32
11	36	14	29	22
12	18	10	14	20
17	13	20	94	16
18	22	15	18	909
19	31	21	15	500
20	18	12	29	33
27	19	10	14	15

Number of p.f.c. in modified, direct Jerne technique after ten oral immunizations with SRBC (each 5 × 10⁸ cells) and one intravenous immunization with 4 × 10⁸ SRBC.

Table 1 compares the number of plaque-forming cells (p.f.c.) before and after oral immunization. The p.f.c. reached a peak in the spleen on the fourth day after intravenous immunization, slowly decreasing on the following days. In the intestines of the same group the number of p.f.c. was above the normal background on the seventh day. An increase in p.f.c. was also found on the seventh day after oral immunization.

In contrast to intravenous immunization, however, a further larger increase in p.f.c. was observed in the intestine on the eighteenth and nineteenth days. In all control animals 0-8 p.f.c./10⁶ were detected in the intestine and spleen by the

Jerne technique with SRBC. These results were fully reproducible in four identical series of experiments.

The results show that IgM-forming cells can be demonstrated in the intestinal tissue by the Jerne technique after both parenteral and oral antigen administration, but the immune response induced by oral immunization differs from that seen after intravenous immunization. It is characteristic of the immune response demonstrated by the modified Jerne technique that the occurrence of IgM-forming cells in the lamina propria of the intestine is delayed and may happen only on the seventh day. The immune response in the lamina propria after oral immunization obviously follows kinetics other than those of the immune response of the general immune apparatus.

It is possible, however, that the lamina propria contains two IgM-forming cell populations. The first (manifest by an increase in the number of p.f.c. on the seventh day) may be stimulated orally or parenterally. The second population (manifest by increase in the p.f.c. number on the eighteenth or nineteenth day) shows delayed onset with antibody formation and reacts only to antigen stimulus from the intestinal lumen. The first population must be assigned to the immune apparatus, for the spleen reacts in the same manner, whereas the second one belongs probably to the immune system of the mucous membranes.

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- ¹ Tomasi, jun., T. B., Tan, E. M., Solomon, A., and Prendergast, R. A., *J. Exp. Med.*, **121**, 101 (1965).
- ² Crabbé, P. A., Carbonara, O. A., and Heremans, J. F., *Lab. Invest.*, **14**, 235 (1965).
- ³ O'Daly, J. A., and Cebra, J. J., *Protides of the Biological Fluids* (edit. by Peeters, H.), 205 (Proc. Sixteenth Coll., Bruges, 1968).
- ⁴ Steffen, C., *Allgemeine Experimentelle Immunologie und Immunopathologie sowie Ihre Klinische Anwendung*, 614 (Thieme, Stuttgart, 1968).
- ⁵ Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell-bound Antibodies* (edit. by Amos, B., and Koprowski, H.), 109 (Wistar Institute Press, 1963).
- ⁶ Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).
- ⁷ Crabbé, P. A., Nash, D. R., Bazin, H., Eyssen, H., and Heremans, J. F., *J. Exp. Med.*, **130**, 723 (1969).

Induction of Immunity of Lambs to a Larval Cestode by Diffusible Antigens

THE intermediate hosts of taeniid cestodes are known to develop strong immunity after a natural infection, and attempts have been made to induce this immunity artificially by the use of a number of antigen preparations¹⁻⁸. In general, it has been found that useful immunity is stimulated only by living parasites, which has led to speculations that the metabolic excretions and secretions released by the parasite during development contain the antigens responsible for inducing protective immunity. We have carried out an experiment using lambs infected with *Taenia ovis* which provides support for this hypothesis.

We used three groups of five cestode-free and nematode-free lambs, 4 weeks old, and eggs of *T. ovis* which had been collected from experimentally infected dogs and carefully washed with antibiotic solutions. The eggs were artificially hatched and the embryos were activated by Heath's⁹ method. Activated embryos were placed in sterile 14 mm diffusion chambers ('Millipore', pore size 0.22 μ m), and the sealed cham-

bers were implanted into the peritoneal cavity of the lambs in two of the three groups. The third group served as the control.

Chambers were removed from one of the implanted groups after 1 week, and from the other group after 3 weeks. Development of *T. ovis* embryos had occurred in all of the diffusion chambers. By 3 weeks the larvae had reached an average size of 1.8 $\times$ 1.7 mm, indicating a rate of growth similar to that found in natural taeniid infections¹⁰⁻¹³.

Two weeks after removal of the chambers, all lambs were exposed orally to 2,000 *T. ovis* eggs. Six weeks later the lambs were killed and the striated muscle, including heart, diaphragm and tongue, was examined for *Cysticercus ovis*. The carcass muscle was removed from the bone and cut into 3 mm slices so that all cysts could be detected. Cysts of normal appearance and those showing evidence of degeneration were estimated separately.

Table 1 Numbers of Developing and Degenerating *C. ovis* in the Muscle of Lambs killed 6 Weeks after Oral Infection with 2,000 *T. ovis* Eggs

Group	Type	Mean No. of larvae from challenge infection		
		Total	Developing	Degenerating
1	Control	113.8	62.8	51.0
2	1 week implantation	0.0	0.0	0.0
3	3 week implantation	1.0	0.0	1.0

Table 1 shows that lambs developed absolute immunity after only 1 week of exposure to developing *T. ovis* larvae. Furthermore, this immunity was stimulated by diffusible antigen(s) released from the parasite; no direct contact between host cells and parasites was necessary. The numbers of larvae developing in the diffusion chambers in each sheep varied from 15 to 129. As few as fifteen developing *C. ovis* were able therefore to produce sufficient antigen to immunize a lamb. Antigen was effective parenterally; an intestinal migration of larvae was not necessary to induce resistance to early establishment of a challenge infection.

By applying *in vitro* cultivation methods, such as those of Heath⁹, it may be possible to collect the "functional" antigens. These antigens may prove useful for artificial immunization of the intermediate host, or in providing antigens of greater specificity for the diagnosis of cestode infections in man and animals.

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- ¹ Miller, jun., H. M., *J. Prev. Med.*, **5**, 429 (1931).
- ² Miller, jun., H. M., *Proc. Soc. Exp. Biol.*, **29**, 1125 (1932).
- ³ Miller, H. M., and Kerr, K. B., *Proc. Soc. Exp. Biol.*, **29**, 670 (1932).
- ⁴ Campbell, D. H., *Amer. J. Hyg.*, **23**, 104 (1936).
- ⁵ Turner, E. L., Dennis, E. W., and Berberian, D. A., *J. Parasitol.*, **23**, 43 (1937).
- ⁶ Gemmell, M. A., *Immunology*, **7**, 489 (1964).
- ⁷ Gemmell, M. A., *Exp. Parasitol.*, **26**, 58 (1969).
- ⁸ Gemmell, M. A., and Soulsby, E. J. L., *Bull. Wild Hlth Org.*, **39**, 45 (1968).
- ⁹ Heath, D. D., thesis, Australian National Univ. (1970).
- ¹⁰ Crusz, H., *J. Helminth.*, **22**, 179 (1948).
- ¹¹ Hutchison, W. M., *J. Parasitol.*, **44**, 574 (1958).
- ¹² Bilquees, F. M., *Austral. J. Zool.*, **17**, 487 (1969).
- ¹³ Singh, B. B., and Rao, B. V., *Ceylon Vet. J.*, **15**, 121 (1969).

Transplantation Antigens in Rhesus Monkeys

WE read your interesting editorial¹ dealing with our work on the major transplantation antigens of rhesus monkeys, the RhL-A system. Your communication ends: "The previously held concept of H-2 genetic structure is now very much open to doubt. If further work shows that the RhL-A system also consists of two segregant series of antigens it is bound to undermine even more the concept of H-2 as a system of multiple, pseudoallelic antigens".

It might be worth mentioning that in the months that passed since we submitted our communication, experimental evidence has been obtained which strongly suggests that there are indeed two segregant series of alleles which determine the antigens of the RhL-A system. Further, our work on chimpanzee cells revealed that the principal leucocyte antigens of that primate species may also be governed by one genetic system, tentatively called ChL-A³. Recent evidence obtained in cross-species typing between chimpanzee and man indicated that some of these specificities are closely related to human HL-A antigens of the first or "LA" series of alleles, while several other chimpanzee antigens resemble specificities of the second or "Four" series of HL-A (this last group of chimpanzee specificities exhibited a phenotypic distribution in 200 animals, suggesting their determination by a series of allelic genes; the others did not fit into the same allelic series). The data therefore suggest that the tissue antigens of chimpanzees may also be determined by two series of closely linked genes³.

The opinion expressed in your editorial that, as in the human HL-A system, two linked series of allelic genes may determine the major histocompatibility antigens also in other mammalian species seems to be corroborated by our preliminary data for monkeys and chimpanzees.

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¹ *Nature*, **230**, 279 (1971).

² Balner, H., Gabb, B. W., Dersjant, H., Van Vreeswijk, W., and van Rood, J. J., *Nature New Biology*, **230**, 177 (1971).

³ Balner, H., et al., *Transplantation*, **11**, 309 (1971).

⁴ Balner, H., et al., *Transplant. Proc.* (in the press).

Communication between Cells of Different Type

MANY types of cells have membrane junctions so organized as to allow the passage of molecules from the interior of one cell to another with little loss to the outside¹⁻⁶. At least in some cases, a large part if not all of the surface membrane seems to be capable of this organization, for if the junction is broken and the cell pair rejoined at some other place, the system can become fully communicating within seconds or minutes^{7,8}. Evidently the membranes must undergo profound structural changes in such a way that previously impermeable surface regions become highly permeable. This implies a close spatial matching of the joining regions on the two membranes. The question arises whether such matching can be effected between membranes that differ from each other. To find something out about this problem, we have paired cultured cells from different organs and species, measuring the communication between cells electrically.

We have used rabbit lens epithelial cells (lens)⁹, 3T3 mouse fibroblasts transformed by simian virus 40 (SV40-3T3)¹⁰, hamster kidney fibroblasts (BHK-21)¹¹, a polyoma-transformed derivative of BHK-21 (P γ Y), a mutant of P γ Y, P γ Y-

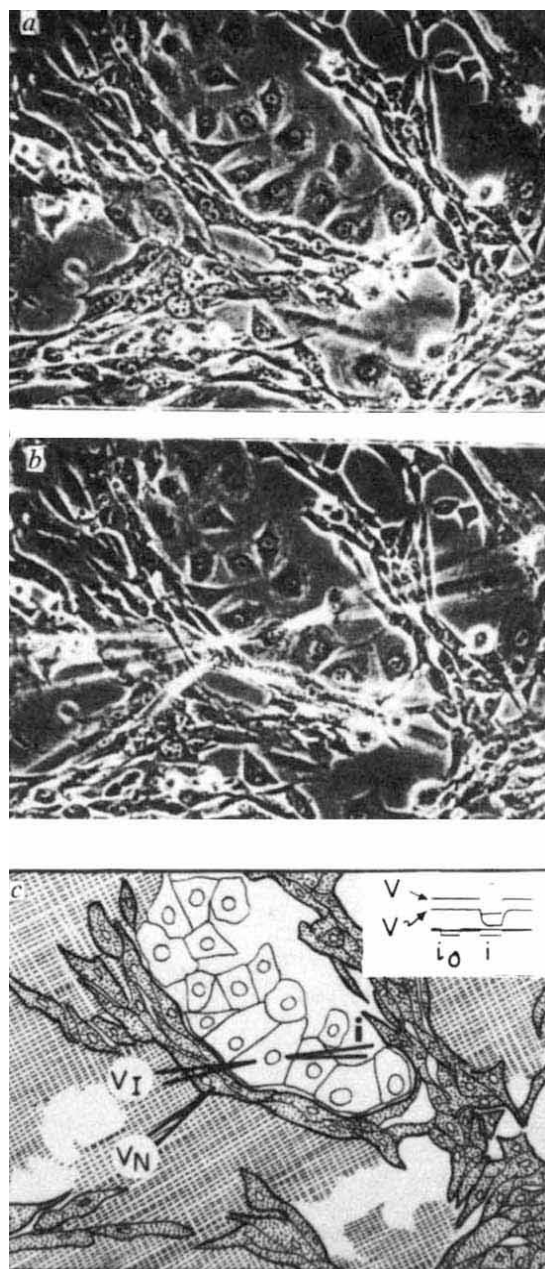


Fig. 1 a, A co-culture of hamster kidney fibroblasts (BHK-21) and rat liver cells, shown in b with 3 microelectrodes in intracellular position for measurement of coupling. On the tracing below, the BHK cells are dotted and the liver cells clear. A current-delivering (i) and voltage-recording (V_1) electrode are inside a liver cell and a second voltage-recording electrode V is in a BHK cell. Inset, an oscilloscope sample record from a measurement on a similar co-culture. (The first current pulse (i_0) is delivered with a fourth electrode outside the cells.) $i = 1 \times 10^{-8}$ A; 100 ms; $V_1 = 25$ mV.

TG-TGR (TGR)¹² and rat liver epithelial cells (liver)¹³. The first two cell types were grown in Eagle's medium¹⁴ as modified by Vogt and Dulbecco¹⁵ (ED medium) with 15% calf serum, the next two in BHK-21 medium¹¹ with 10% calf serum and 10% tryptose phosphate broth, and the last one in ED medium with 10% foetal calf serum. All cell mixtures were grown in ED medium with 15% calf serum. The cell cultures were kept in plastic Petri dishes at 37° C and equilibrated at pH 7.3 with a moist CO₂-air mixture.

Intercellular communication was measured by injecting an electrical current (i) into one cell (1) of a group of cells in visible contact with each other and measuring the resulting voltages in this cell (V_1) and another cell (V_N) of the group, contiguous with cell 1 or separated by one or two inter-

vening cells. The ratio V_N/V_1 provided the index of communication¹ (Fig. 1). During the measurement the culture dishes were at room temperature and exposed to air and were perfused continuously with fresh medium to keep the pH constant.

In all combinations tested, the cells were clearly identifiable in phase contrast, especially when the cells of different kinds were not completely interspersed but formed groups of their own type. The measurements were, therefore, done preferentially between members at the boundary of two such cell groups.

Table 1 Electrically Measured Communication between Different Cell Types in Culture

Cell type combined with	Lens	Liver	BHK-21	TGR	SV40-3T3
Lens	+	+	+	+	+
Liver		+	+	+	
BHK-21			+		
TGR				+	

All combinations tested showed an index of communication of at least 0.2, but mostly higher (+). Some combinations were not tested because unambiguous identification of the cells was not possible (no sign).

The results are summarized in Table 1. All cell combinations examined showed electrical coupling. The lens/liver cell junction, for instance, had a communication index V_N/V_1 of the order of 0.4. The lens/lens and liver/liver junctions had indices of the order of 0.8. The communication indices varied greatly within a given cell combination and between various combinations, probably reflecting variations of the number and area of cell junctions and other factors in the geometry of current flow and perhaps also the differences in the quality of the junctions. Communication indices of at least 0.2 could be obtained in all cell combinations.

The maximum V_N/V_1 values for the various cell combinations were: lens/lens, 0.88; lens/liver, 0.41; lens/TGR, 0.38; lens/SV40-3T3, 0.23; liver/liver, 0.73; liver/BHK-21, 0.88; liver/TGR, 0.85; BHK-21/BHK-21, 0.37; P γ Y/P γ Y, 0.44. In the case of the lens/BHK-21 combination, mostly transfer resistances (V_N/i) were measured; the maximum value was $2.1 \times 10^5 \Omega$.

These results show that cultured cells from different organs and different organisms can establish communicating junctions among each other. Evidently for the formation of communicative junction the joining membranes need not be entirely equal. The membranes of the cells we used may have been more similar to each other than those of their counterparts inside the organism because of dedifferentiation in culture. But the cells are clearly genetically different and the membrane proteins are therefore probably not identical. In the case of transformed cells, differences in the surface structure have been shown immunologically^{16,17}. Thus the capacity to form communicating junctions seems to be a basic and general characteristic of membranes, at least in mammalian cells. It is interesting in this connexion that a group of small proteins has been identified as a major membrane component common to various cells of different type and from different species¹⁸.

With certain sponges, however, pairing of a cell of one species (*Microciona*) with a cell of another species (*Halysclona*) does not result in formation of communicative junction, although pairs of cells from the same species make junctions readily⁷. This correlates with the adhesive properties of these cells^{19,20}. It may be relevant here that these two animals are much farther apart on the evolutionary scale than the mammalian cells here tested.

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- Loewenstein, W. R., and Kanno, Y., *J. Cell. Biol.*, **22**, 565 (1964).
- Loewenstein, W. R., *Ann. NY Acad. Sci.*, **137**, 441 (1966).
- Pappas, G. D., and Bennett, M. V. L., *Ann. NY Acad. Sci.*, **137**, 495 (1966).
- Furshpan, E. J., and Potter, D. D., *Curr. Topics Develop. Biol.*, **3**, 95 (1968).
- Loewenstein, W. R., in *The Emergence of Order in Developing Systems* (edit. by Locke, M.), *Develop. Biol.*, **18**, SP.2, 151 (1968).
- Payton, B. W., Bennett, M. V. L., and Pappas, G. D., *Science*, **166**, 1641 (1969).
- Loewenstein, W. R., *Develop. Biol.*, **15**, 503 (1967).
- Ito, S., and Loewenstein, W. R., *Develop. Biol.*, **19**, 228 (1969).
- Shapiro, A. L., Siegel, I. M., Scharff, M. D., and Robins, E., *Invest. Ophthalmol.*, **8**, 393 (1969).
- Todaro, G. J., Green, H., and Goldberg, B. D., *Proc. US Nat. Acad. Sci.*, **51**, 66 (1964).
- MacPherson, I. A., and Stoker, M. G. P., *Virology*, **16**, 147 (1962).
- Subak-Sharpe, H., Burk, R. R., and Pitts, J. D., *J. Cell Sci.*, **4**, 353 (1960).
- Borek, C., Higashino, S., and Loewenstein, W. R., *J. Membrane Biol.*, **1**, 274 (1969).
- Eagle, H., Oyama, V. I., Lory, M., and Freeman, A. E., *J. Biol. Chem.*, **226**, 191 (1957).
- Vogt, M., and Dulbecco, R., *Proc. US Nat. Acad. Sci.*, **46**, 365 (1960).
- Habel, K., *Cold Spring Harbor Symp. Quant. Biol.*, **27**, 433 (1962).
- Todaro, G. T., Habel, K., and Green, H., *Virology*, **27**, 179 (1965).
- Laico, M. T., Ruoslahti, E. I., Papermaster, D. S., and Dreyer, W. J., *Proc. US Nat. Acad. Sci.*, **67**, 120 (1970).
- Moscona, A. A., *Proc. US Nat. Acad. Sci.*, **49**, 742 (1963).
- Humphreys, T., *Develop. Biol.*, **8**, 27 (1963).

Vasodilator Nerve Fibres to the Submaxillary Gland of the Cat

IN 1851 Claude Bernard¹ presented the first evidence for the existence of vasoconstrictor nerves in the cervical sympathetic nerve to the blood vessels of the rabbit's ear. In 1858 he demonstrated² that there was a dual neurovascular regulation in the submaxillary gland of the dog by showing, that whereas electrical stimulation of the sympathetic nerve reduced the venous outflow from the gland, stimulation of the parasympathetic (chorda tympani) greatly increased it. He concluded, therefore, that there were vasodilator, as well as vasoconstrictor, nerves and that this afforded a mechanism whereby the blood flow through the gland was regulated locally, independent of alterations in systemic blood pressure. He regarded this as a further example of his principle of homeostasis.

For many years afterwards it was assumed that vasodilator and vasoconstrictor nerves existed in the submaxillary gland, until the studies of Barcroft and Piper³ in 1912 suggested that the secretory metabolism of the salivary gland was in some way responsible for the accompanying vasodilatation. Barcroft⁴ did not, however, dismiss the possible role of vasodilator nerves and he stated that "under normal circumstances dilatation may be instituted by dilator fibres and maintained by metabolic products". Later workers also thought that vasodilator nerve fibres to the submaxillary gland existed⁵⁻⁷.

Until about 1960, it was widely accepted by physiologists that vasodilator nerve fibres existed, although an additional but somewhat vague role of "metabolites" was also assumed. But as a result of recent experiments⁸⁻¹⁰, it has become widely assumed¹¹⁻¹³ that such fibres do not exist. Hilton and Lewis concluded, from experiments on blood flow in the submaxillary gland of the cat, that the release of kallikrein from the secretory cell during activity could be the cause of the vasodilatation and that there were, therefore, no vasodilator nerve fibres.

Lundberg¹⁴ inserted intracellular electrodes in secretory cells of this gland and observed a hyperpolarization of 5–20 mV with a latency of 200–400 ms after applying single shocks to the chorda tympani nerve. Creed and Wilson¹⁵ performed similar experiments and observed latencies varying from 252–420 ms, with a mean of approximately 300 ms, when the chorda tympani nerve was stimulated at a frequency of 20 pulses s⁻¹. We have repeated and confirmed these observations.

Our experiments were designed to avoid mechanical delays in measuring the latency between the onset of the vasodilatation after nerve stimulation and compare it with the corresponding latency of the change in membrane potential of the secretory cells in the submaxillary gland. We used a forced-convection flowmeter¹⁶ to minimize these mechanical delays. This instrument is of the thermal type, designed to record rapidly a wide range of rates of flow. The sensor device is a thermistor kept at constant temperature as the cooling blood flows past it. The voltage which controls the amount of power required to maintain the thermistor at a constant temperature is a function of the velocity of the flow of fluid. A flow-insensitive reference thermistor compensates for any changes in blood temperature, and both are contained in a glass probe approximately 2–3 cm in length and 1–1.5 mm in internal diameter.

Cats weighing 2–4 kg were anaesthetized with chloralose (80 mg kg⁻¹ given intravenously). The blood flow through the submaxillary gland was measured by inserting the thermistor probe into an external jugular vein by a double cannulation; all veins draining into the external jugular were ligatured except those from the submaxillary gland. Heparin (10 mg kg⁻¹, given intravenously) was given at the beginning of the experiment with further doses if necessary. Nerve stimulation, blood pressure measurements and collection of saliva were carried out as described previously¹⁷. A prompt flow of saliva after chorda stimulation was assumed to indicate an undamaged nerve supply. The pen recorder (Sanborn) noted the onset and duration of nerve stimulation automatically.

In all experiments, the latency between stimulation of the chorda tympani nerve (7 V, 20 per s) and the onset of vasodilatation was less than in previous experiments using drop-counters^{22,23}. Table 1 summarizes the results obtained in experiments on five cats.

Table 1 Latencies between Stimulation of the Chorda and Onset of Vasodilatation

Experiment	Shortest latency
Cat 1	800 ms
Cat 2	900 ms
Cat 3	550 ms
Cat 4	350 ms
Cat 5	650 ms

The latency values shown in Table 1, although short, are probably longer than the true values. For example, because veins are collapsible structures, a change in blood flow in one region does not produce an instantaneous change in distal regions. Measurements on flow in the external jugular vein *in vitro* showed that the delay between very small pressure changes (about 2 mm Hg) in the submaxillary vein and flow changes at the probe (about 3 cm away) was of the order of 100 ms. The flow rates measured in this model *in vitro* were similar to the basal rates observed *in vivo*.

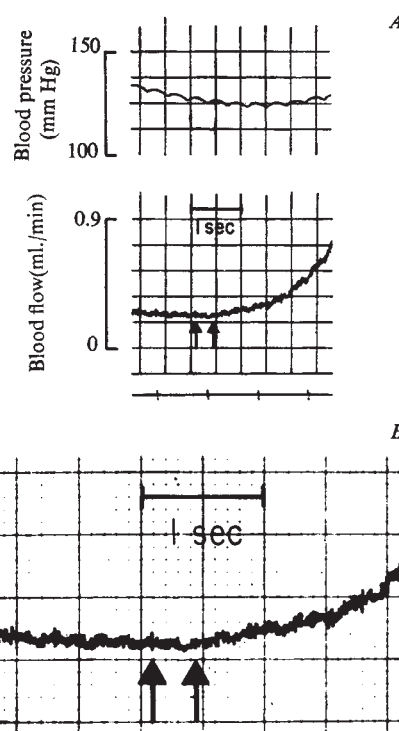


Fig. 1 Blood flow changes in the submaxillary gland of a cat, caused by stimulation of the chorda tympani nerve for 3 s. **A**, Blood flow changes and the systemic blood pressure. Arrows indicate the beginning of nerve stimulation and the onset of vasodilatation, respectively. **B**, An enlarged portion of **A** showing only the blood flow changes.

Fig. 1 shows a record of blood flow changes in the submaxillary gland caused by 3 s of stimulation of the chorda tympani nerve. Fig. 1A shows that although the arterial blood pressure remained constant, there was a very rapid increase in blood flow through the gland. The first arrow marks the start of stimulation, and the second the onset of vasodilatation. The latency between nerve stimulation and vasodilatation was approximately 350 ms (Fig. 1B).

According to Creed and Wilson¹⁵, the mean latency between nerve stimulation and hyperpolarization of the secretory cell was approximately 300 ms at a stimulation frequency of 20 pulses per second. We have repeated and confirmed their observations in similar experiments. Approximately 60 ms of this latency can be accounted for by nerve conduction and ganglionic delay, while the greater part is attributable to the process of activation of the secretory cell by acetylcholine, and to the initiation of hyperpolarization. Because our shortest latency between nerve stimulation and onset of vasodilatation was 350 ms, the kallikrein-kinin system is not involved in the initiation of this vasodilatation caused by stimulation of the chorda tympani nerve. Even in those experiments with the longest latencies (for example, 900 ms) it is most unlikely that there would be time for the activation of the secretory cell by acetylcholine, the secretion of kallikrein into the interstitial fluid, the enzymatic release of kallidin and the final vasodilator action of kallidin, either by some direct action on vascular smooth muscle, or by the longer pathway of a veno-arteriolar chemosensory reflex.

Our present experiments provide direct physiological evidence to indicate the existence of vasodilator nerve fibres ending close to the smooth muscle cells of blood vessels in the submaxillary gland. This is consistent with the available histological evidence¹⁸⁻²¹. Gautvik²² has also reached this conclusion after observing longer latencies (namely 1–6 s) between stimulation of the chorda tympani nerve and the onset of vasodilatation. Like Bhoola *et al.*²³, who observed latencies of 2–4 s,

Gautvik used a drop-counter, which involves long mechanical delays in the measurement of flow rate. Whereas Gautvik concluded that dilator nerve fibres initiate the vasodilatation, he suggested that it is "maintained" by the "metabolite", kallikrein. From our experiments we cannot assess the contribution of kallikrein. Previous experiments of Schachter and his colleagues^{17,23,24} indicate that dilator nerve fibres can account for most of the vasodilatation observed.

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- ¹ Bernard, C., *CR Soc. Biol.*, **4**, 163 (1851).
- ² Bernard, C., *CR Acad. Sci.*, **47**, 245 (1858).
- ³ Barcroft, J., and Piper, H., *J. Physiol.*, **44**, 359 (1912).
- ⁴ Barcroft, J., *The Respiratory Function of the Blood* (University Press, London, 1914).
- ⁵ Bayliss, W. M., *The Vaso-motor System* (Longmans, New York, 1923).
- ⁶ Dale, H. H., and Gaddum, J. H., *J. Physiol.*, **70**, 109 (1930).
- ⁷ Babkin, B. P., *Secretory Mechanism of the Digestive Glands* (Hoeber, New York, 1950).
- ⁸ Hilton, S. M., and Lewis, G. P., *J. Physiol.*, **128**, 235 (1955).
- ⁹ Hilton, S. M., and Lewis, G. P., *J. Physiol.*, **129**, 253 (1955).
- ¹⁰ Hilton, S. M., and Lewis, G. P., *J. Physiol.*, **134**, 471 (1956).
- ¹¹ Mountcastle, V. B., *Textbook of Medical Physiology* (Mosby, St Louis, 1968).
- ¹² Davenport, H. W., *Physiology of the Digestive Tract* (Year Book Med. Pub., Chicago, 1966).
- ¹³ Selkurt, E. E., *Physiology* (Little, Boston, 1966).
- ¹⁴ Lundberg, A., *Acta Physiol. Scand.*, **35**, 1 (1955).
- ¹⁵ Creed, K. E., and Wilson, J. A. F., *Austral. J. Exp. Biol. Med. Sci.*, **47**, 135 (1969).
- ¹⁶ Karpinski, E., thesis, Univ. Alberta (1971).
- ¹⁷ Beilenson, S., Schachter, M., and Smaje, L. H., *J. Physiol.*, **199**, 303 (1968).
- ¹⁸ Kuntz, A., and Richins, C. A., *J. Comp. Neurol.*, **85**, 21 (1946).
- ¹⁹ Snell, R. S., and Garrett, J. R., *Z. Zellforsch.*, **48**, 201 (1958).
- ²⁰ Garrett, J. R., *J. Roy. Microsc. Soc.*, **86**, 1 (1966).
- ²¹ Garrett, J. R., *J. Roy. Microsc. Soc.*, **86**, 15 (1966).
- ²² Gautvik, K., *Acta Physiol. Scand.*, **79**, 174 (1970).
- ²³ Bhoola, K. D., Morley, J., Schachter, M., and Smaje, L. H., *J. Physiol.*, **179**, 172 (1965).
- ²⁴ Morley, J., Schachter, M., and Smaje, L. H., *J. Physiol.*, **187**, 595 (1966).

Specific Appearance of Sympathetic Cholinergic Vasodilatation in Muscles during Conditioned Movements

THE anticipation of movement might increase blood flow to the skeletal muscles, and it is usually assumed that this adaptive function is performed by the sympathetic cholinergic system¹. This consists of a series of cerebral relays connecting the motor cortex, the hypothalamus, and the mesencephalic periaqueductal grey to sympathetic postganglionic neurones exerting an atropine-sensitive dilating action on the vasculature of skeletal muscles^{2,3}. Although it has been demonstrated that the sympathetic cholinergic system can be conditioned^{4,5}, there is no clear evidence of its relationship to movement, nor any basis for suggesting that its function is specific, limited to active muscles (as would be expected should it subserve muscle exercise²), or more diffuse and indiscriminate (as might be advantageous in emotional behaviour⁶). The study described here was designed to define more precisely whether the sympathetic cholinergic system can exert a specific and motor-related action.

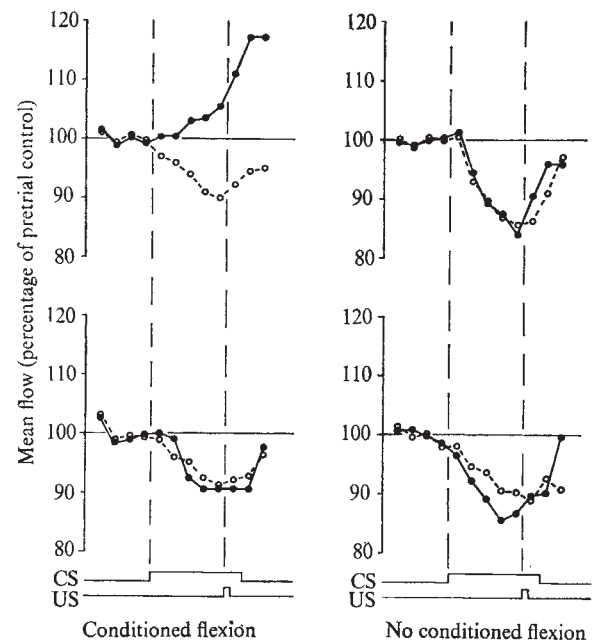


Fig. 1 Changes in external iliac blood flow to the trained (●) and untrained (○) hindlimbs, during conditioning trials in which the trained limb gave conditioned flexion (left column) and in which there was no conditioned flexion (right column). Two upper sections (no drugs): trials before, and two lower sections (methylatropine): trials after injection of 0.5 mg/kg of the cholinergic blocking agent. In all the curves each point represents the mean of 1 s measurements performed during forty similar trials (ten trials for each of four cats), expressed as percentage changes with reference to baseline values measured while the animal was quiet before the trial. The vertical interrupted lines indicate the beginning of conditioned and unconditioned stimuli. The duration of stimuli is indicated at the bottom of the figure.

Cats were pre-trained, using classical conditioning, with a 500 Hz tone of moderate intensity which signalled the onset of a 1–3 mA shock lasting 400 ms to one of the two hindlimbs. Each cat always received the shock to the same limb. The interval between conditioned stimulus onset and the unconditioned stimulus was 1 s in the initial conditioning trials, and was lengthened to 5 or 6 s after several days training. Those animals which learned (after about 200 trials) to give a relatively specific unilateral leg flexion response (during the tone) were implanted with Statham electromagnetic flow probes and collapsible snares (used to produce zero flow for calibration) on both external iliac arteries. Electromyograph (EMG) electrodes were also placed in each of the hindlegs. After a week of recovery, a blood pressure cannula was placed either in a side branch of a femoral artery or in one of the carotid arteries.

During behavioural testing the cat, usually lying on the side contralateral to the shocked limb, was under constant visual observation. Instantaneous blood flows to the two hindlimbs and their 1 s integrals were displayed on a 'Grass P7' polygraph together with the instantaneous and mean (integrated) blood pressure. Conditioned flexion movements were recorded by a signal marker triggered by the observer and with the EMG electrodes.

Fig. 1 summarizes the data from the four cats in which training and surgery were successful. The upper left quarter shows the mean percentage changes in flow from the pre-trial levels during trials in which the animals showed a relatively specific conditioned flexion response of only the trained limb. Flow increased only in the trained (that is flexed) limb (filled circles), while the contralateral limb had a net decrease in flow (open circles). The unilateral increase in flow began at the moment of flexion. During trials when no conditioned flexion occurred (Fig. 1, upper right), flow to both limbs was reduced equally. Blood pressure changes during both types of trial

were slight, and computation of vascular conductances (by dividing the blood flow by the mean arterial pressure) yielded curves exactly paralleling those in Fig. 1, thus showing that flow increases and decreases always meant vasodilatation and vasoconstriction, respectively. Analyses of variance⁷ indicated that each of these vasomotor changes was highly significant ($P < 0.01$).

The two lower sections of Fig. 1 illustrate the changes in flow during similar trials following the administration of methyl-atropine (0.5 mg/kg), a cholinergic blocking agent which does not cross the blood-brain barrier^{8,9}. The flow increase and vasodilatation previously observed in the trained limb during conditioned flexion were abolished and the two limbs had a parallel vasoconstriction. The difference between the change in flow in the trained limb before and after atropine was significant ($P < 0.05$).

These experiments demonstrate that the sympathetic cholinergic system is closely connected to movement, and that when testing conditions are arranged so that a highly specific conditioned motor response develops, this portion of the sympathetic system also acts in a highly specific fashion, parallel to the skeletal motor system. Indeed cholinergic vasodilatation appeared in these experiments only when conditioned movements occurred, and only in the responding leg. Because we purposefully induced very short conditioned movements, it should not be surprising that a large proportion of the conditioned vasodilatation was cholinergic in our experiments. Whenever stronger, prolonged flexions occurred an atropine-resistant, probably metabolic, component of the vasodilatation developed.

In our conditions there was little evidence that the cholinergic vasodilatation, although undoubtedly connected with movement, acted in a preparatory fashion, but it seemed to shunt blood to skeletal muscles only as they were used, while a diffuse vasoconstriction was associated with immobile expectation. This might help to explain why only vasoconstriction is seen in anticipation of fighting¹⁰, when skeletal movements are absent or minimal.

The high degree of specificity here demonstrated for the cholinergic portion of the sympathetic system has two further implications. The first is that it indirectly supports evidence¹¹ that by instrumental conditioning animals can learn to control other parts of the autonomic nervous system. There is a second, far reaching implication. It seems to be reasonable to assume that, in a conditioning situation such as we have studied, the sympathetic cholinergic neurones would be activated through their central relays. It follows that these central structures can operate as a highly organized motor system, a degree of specificity not usually attributed to regions such as the hypothalamus and the mesencephalic periaqueductal grey.

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¹ Uvnäs, B., *Handbook of Physiology*, Section 1, *Neurophysiology*, 2, 1131 (Amer. Physiol. Soc., Washington, 1960).

² Eliasson, S., Folkow, B., Lindgren, P., and Uvnäs, B., *Acta Physiol. Scand.*, 23, 333 (1951).

³ Lindgren, P., *Acta Physiol. Scand., Suppl.*, 35, 121 (1955).

⁴ Abrahams, V. C., Hilton, S. M., and Zbrożyna, A., *J. Physiol.*, 171, 189 (1964).

⁵ Bolme, P., and Novotny, J., *Acta Physiol. Scand.*, 77, 58 (1969).

⁶ Abrahams, V. C., Hilton, S. M., and Zbrożyna, A., *J. Physiol.*, 154, 491 (1960).

⁷ Snedecor, G. W., and Cochran, W. G., *Statistical Methods* (Iowa State University Press, Ames, 1967).

⁸ Herz, A., Teschemacher, H., Hofstetter, A., and Kurz, H., *Intern. J. Neuropharmacol.*, 4, 207 (1965).

⁹ Guazzi, M., Mancina, G., Kumazawa, T., Baccelli, G., and Zanchetti, A., *Cardiovascular Res.*, 3, 265 (1968).

¹⁰ Adams, D. B., Baccelli, G., Mancina, G., and Zanchetti, A., *Nature*, 220, 1239 (1968).

¹¹ Miller, N. E., *Science*, 163, 434 (1969).

Isolation of Specific Macromolecules required for Adhesion of Mouse Tumour Cells

THE adhesive properties of cell surfaces which seem to determine many morphogenetic processes¹ and which may be important in malignancy² must reflect the biochemical characteristics of the cell surfaces. There have been attempts to isolate the macromolecules concerned by assaying for substances required for cell adhesion³, and one of us⁴ has described a promising experimental system for such studies. Trypsin-dissociated cells from an ascites-grown form of a mouse teratoma (teratocarcinoma, embryonal carcinoma)^{5,6} require L-glutamine in order to synthesize amino-sugars and to reaggregate in a solution of glucose and balanced salts, most probably because the amino-sugars are necessary for the formation of the complex carbohydrates involved in cell adhesion⁴. This communication describes experiments in which the amino-sugars are replaced by macromolecular substances in the ascites fluid in which the teratoma grew.

A strain of 129 mouse teratoma which grew as cell clusters termed "embryoid bodies"⁴⁻⁶ was used and handled as previously described⁴. Cells were grown in 129/J mice, collected from the ascites fluid, incubated in calcium-magnesium free solution and in 0.7% trypsin, dissociated, washed again and resuspended in HEPES buffered Hanks solution (pH 7.5) with 10 µg/ml. DNAase at a concentration of about 6×10^5 single teratoma cells/ml. Aliquots of this suspension were diluted by half with Hanks or various test preparations and 0.25 ml. aliquots were placed in one dram screw cap vials at 37° C on a gyratory shaker running at 68 r.p.m. with a 4-5/8 inch diameter of rotation⁷. At various times, vials were placed on ice, diluted with 10 ml. of 'Isoton' (Coulter Electronics) and counted with a model B Coulter counter with a 0.5 ml. manometer, using a setting of 1/amplication=8 and 1/aperture current=0.707 and a window of 20 to 100. These settings counted 80% of the single cells but excluded most aggregates and cellular debris. Aggregation was measured as the disappearance of single cells^{4,8,9}, which in all the conditions described here is caused by aggregation and not cell lysis^{4,9}. Replicate vials gave values which differed by an average of 4%.

Teratoma cells which failed to aggregate in Hanks balanced salts solution and slowly reaggregated in the presence of L-glutamine aggregated rapidly when teratoma ascites fluid centrifuged at 30,000g for 15 min was added to Hanks solution (Fig. 1). The ascites fluid contained a factor more efficient than L-glutamine in promoting aggregation. Aggregation increased linearly with concentration at low concentrations but it eventually reached a rate which could not be increased by adding more material (Fig. 2). At high concentrations large, visible aggregates were formed after 15-45 min. Different preparations of ascites fluid varied in activity. The most active samples were obtained from mice receiving high initial doses of the tumour. When more than 3 ml. of ascites fluid accumulated in the peritoneal cavity of the mouse, activity often decreased.

It is possible that the factor discovered did not come from the teratoma. For example, it could be a serum protein. But this is unlikely because less than 7% of the teratoma cells aggregated by 60 min in 50% mouse serum (prepared from 129/J

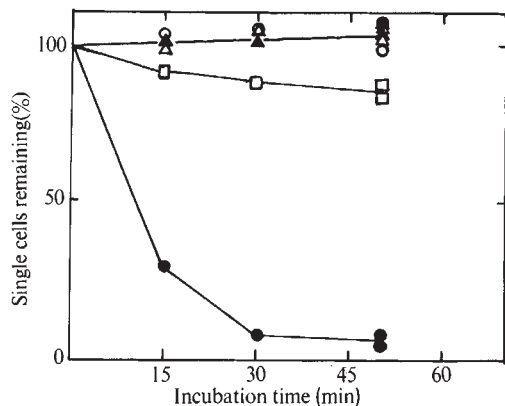


Fig. 1 Specificity of teratoma ascites fluid in promoting aggregation. The cells were suspended in Hanks solution, in Hanks solution containing 6.9 mM L-glutamine or in Hanks solution containing 50% teratoma ascites fluid. Aggregation was determined as the percentage of single cells remaining unaggregated in the standard conditions described in the text. Sarcoma 180 cells, grown in 129/J mice and subjected to the same trypsinization procedure as the teratoma cells, were counted using Coulter counter settings of 1/amplification=16 and 1/aperture current=0.707 and a window of 20-100. The window included nearly all the sarcoma cells as a distinct single peak. The symbols used in the figure are as follows. ○, Teratoma cells in Hanks alone; ●, teratoma cells in 50% teratoma ascites fluid; □, teratoma cells in 6.9 mM L-glutamine; △, sarcoma 180 cells in Hanks alone; ▲, sarcoma 180 cells in 50% teratoma ascites fluid.

mice), foetal calf serum, calf serum or horse serum (Hyland), and as mentioned here, activity increased with increasing tumour cell inoculum and not with increased fluid quantity. Blood clotting reactions do not seem to be involved in the activity of the factor since 10 USP U/ml. of heparin (Clay Adams) did not inhibit activity. The factor does not seem to be gamma-globulin produced by the host mouse against the tumour cells, for no activity was removed from ascites fluid by extensive precipitation with goat anti-mouse gamma globulin anti-serum.

If the active factor was from the teratoma cells, it might be expected to have a greater effect on the aggregation of teratoma cells than on other cells. It did not affect the aggregation of mouse ascites sarcoma 180 cells grown in 129/J mice (Fig. 1), trypsin dissociated 7 day chick embryo neural retina cells and

cells of the sponge, *Microciona parthena*, dissociated by removal of divalent cations.

The aggregation promoting activity of ascites fluid was removed by the addition of 3×10^{-3} M EDTA but it reappeared when Ca^{2+} was added (Fig. 3). This indicates that its activity, like aggregation in many systems³, depends on divalent cations.

The nature of the active component of the ascites fluid was examined and the data are summarized in Table 1 and Figs. 4 and 5. Activity was not dialysable nor was it affected by freezing and thawing or overnight incubation at 4° C. It remained in solution after centrifugation at 100,000g for 1 h, a treatment which also seems to remove aggregation-inhibiting material in the fluid. Activity was completely lost after heating at 100° C for 30 s. Crystalline deoxyribonuclease and ribonuclease (Sigma) treatment for 10 min at 37° C did not affect activity while similar treatment with crystalline trypsin (Calbiochem) greatly reduced the activity. The active component in the fluid binds to two anionic exchangers, DEAE cellulose (Serva) and 'Ecteola' cellulose (Bio-Rad) but not in any great extent to cationic CM cellulose (Bio-Rad) at pH 7.5 in our standard 0.14 M NaCl, 0.01 M Hepes buffer. The active material is included in 'Sephadex 6B' (Pharmacia) (Fig. 4)

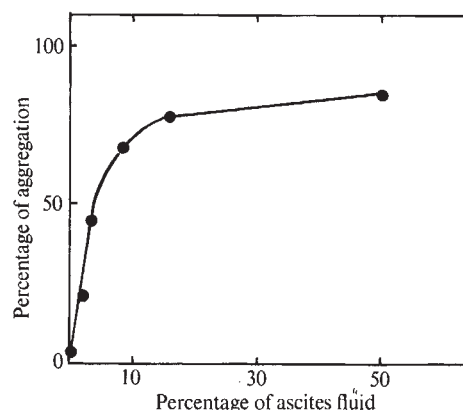


Fig. 2 Effect of concentration of teratoma ascites fluid on the aggregation of teratoma cells. The cells were suspended in Hanks solution containing the indicated concentration of teratoma ascites fluid. Cells were counted after 60 min rotation in standard conditions. Percentage aggregation gives the percentage of cells which have aggregated in the 60 min.

Table 1 Characteristics of the Aggregating Promoting Activity of Teratoma Ascites Fluid

Treatment of ascites fluid	Relative activity* (%)
Dialysis at 4° C against Hepes buffered Hanks solution (pH 7.5) overnight	105
Freezing and thawing	99
Overnight incubation at 4° C	100
Centrifugation at 100,000g for 60 min	164
Heating at 100° C for 30 s	0
Deoxyribonuclease and ribonuclease † incubation at 37° C for 10 min	100
Trypsin incubation at 37° C for 10 min ‡	23
DEAE cellulose adsorption§	22
'Ecteola' cellulose adsorption§	27
Carboxymethyl cellulose adsorption§	90

* Relative activity is given as % aggregation in treated fluid/% aggregation in untreated fluid $\times 100$.

† Ascites fluid (1 ml.) was incubated at 37° C for 10 min with 2 mg of each enzyme.

‡ Crystalline soybean trypsin inhibitor (2 mg/ml.) was added after the 10 min incubation with trypsin.

§ One volume of ascites fluid was mixed with three volumes of washed resin equilibrated with the standard 0.14 M NaCl, 0.01 M Hepes buffer at pH 7.5, and the supernatants were tested for activity.

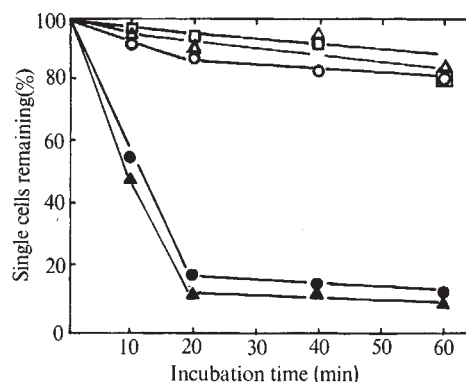


Fig. 3 Requirement of divalent cations for ascites factor activity. Aliquots from the same sample of ascites fluid were treated with 0.10 M EDTA to a final concentration of 3.0×10^{-3} M (○), with EDTA at this concentration followed by the addition after 5 min of 0.10 M calcium chloride to a final concentration of 4×10^{-3} M Ca^{2+} (●) or left untreated (▲); cells incubated in 0.14 M NaCl, 0.01 M pH 7.5 Hepes buffer alone (□); cells incubated in untreated ascites fluid at 4° C (▲). Aggregation was determined as the percentage of single cells remaining unaggregated in the standard conditions described in the text.

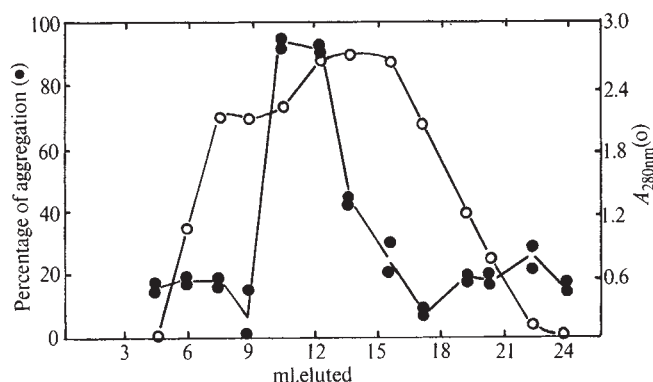


Fig. 4 The elution of protein ($A_{280\text{ nm}}$) and aggregation promoting activity from 'Sephacrose 6B'. The sample (2.8 ml.) was applied to a 25×0.9 cm column of 'Sephacrose 6B' which had previously been equilibrated with 0.14 M NaCl, 0.01 M Hepes, pH 7.5 buffer. The column was eluted with this buffer and fractions were collected and read at $A_{280\text{ nm}}$ (○) and tested for aggregation promoting activity as described in the text. Aggregation in duplicate vials (●) is given as the percentage of single cells aggregated after 60 min in standard conditions.

and sediments at about 12S on a linear 10–40% glycerol gradient (Fig. 5). These results suggest that the active components in the ascites fluid are soluble, negatively charged protein containing macromolecules with a molecular weight of the order of 10^6 .

At 37°C the cells removed the activity from the solution which caused them to aggregate. The factor was effective at 37°C in promoting the aggregation of cells fixed for 15 min in 4% formaldehyde and washed twice by centrifugation; 59% of the fixed cells aggregated in 60 min in the presence of factor while the fixed cells did not aggregate in Hanks alone. These results suggest that the factor attaches to the cells and binds them together without the participation of cellular metabolism. The factor had no effect, however, on the cells at 4°C (Fig. 5) and factor activity was not removed by them at this temperature.

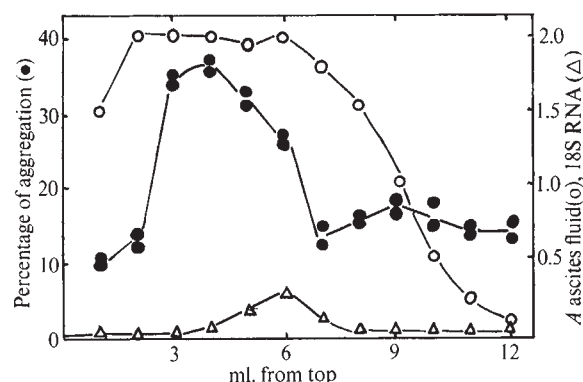


Fig. 5 The protein ($A_{280\text{ nm}}$) profile and aggregation promoting activity of teratoma ascites fluid on a 10–40% glycerol gradient. An active ascites fluid sample (1.5 ml.) and purified 18S sea urchin ribosomal RNA (1.5 ml.) were layered on 10 ml. linear 10–40% glycerol gradients prepared using 0.14 M NaCl, 0.01 M Hepes, pH 7.5 buffer. The two gradients were centrifuged simultaneously at 26,000 r.p.m. for 23.5 h at 4°C in a Beckman model L-2 ultracentrifuge using a type SW 41 rotor. Fractions (1 ml.) were removed from the top of the tubes and the absorbance was read at 280 nm in the case of the ascites fluid (○) or at 260 nm in the case of the RNA marker (Δ). The ascites fluid fractions were dialysed against two 1.5 l. changes of 0.14 M NaCl, 0.01 M pH 7.5 Hepes buffer for 22 h at 4°C and duplicate vials (●) tested for aggregation promoting activity as described in the text. Aggregation is given as the percentage of single cells aggregated in the standard conditions.

Other aggregation promoting factors have been isolated from various normal cells by different techniques^{3,8,10}. The chemical or cellular nature of these various factors has not yet been established but one is thought to be a cell surface glycoprotein³. Since the teratoma cells seem to synthesize glycoproteins or other carbohydrates in order to aggregate⁴, the factor which we have isolated may be the glycoproteins which must be synthesized for aggregation or molecules similar enough to replace them functionally.

Little is known concerning the actual molecules involved in cellular adhesion or about the derangement which may cause the altered characteristics of the surfaces of malignant cells². The active molecules which we have discovered may either carry the biochemical determinants responsible for the altered adhesiveness of these malignant cells or they may be similar to those involved in the adhesion of normal cells but incorporated less well into the tumour cell surface. The second possibility might also explain their release into the ascites fluid. The system we have described will enable a full exploration of these and other possibilities.

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- 1 Townes, P. L., and Holtfreter, J., *J. Exp. Zool.*, **128**, 53 (1955).
- 2 Coman, D. R., *Cancer Res.*, **4**, 625 (1944); Coman, D. R., *Cancer Res.*, **21**, 1436 (1961).
- 3 Humphreys, T., *Devel. Biol.*, **8**, 27 (1963); Humphreys, T., in *The Specificity of Cell Surfaces* (edit. by Davis, B. D., and Warren, L.), 195 (Prentice Hall, Englewood Cliffs, 1967).
- 4 Oppenheimer, S. B., Edidin, M., Orr, C. W., and Roseman, S., *Proc. US Nat. Acad. Sci.*, **63**, 1395 (1969).
- 5 Stevens, L. C., *J. Nat. Cancer Inst.*, **20**, 1257 (1958).
- 6 Kleinsmith, L. J., and Pierce, G. B., *Cancer Res.*, **24**, 1544 (1964).
- 7 Henkart, P., and Humphreys, T., *Exp. Cell Res.*, **63** (in the press).
- 8 Lilien, J. E., *Devel. Biol.*, **17**, 657 (1968).
- 9 Orr, C. W., and Roseman, S., *J. Membrane Biol.*, **1**, 109 (1969).
- 10 Kuroda, Y., *Exp. Cell Res.*, **49**, 626 (1968).

Visual Neurons for Tracking Moving Targets

A CLASS of directionally selective movement detector¹ recorded in the visual system of the privet hawk-moth seems designed in several respects to enable the moth to track a moving target. Although I have found no behavioural evidence that these moths can follow moving objects, the properties of the neurons will be described in the context of visual tracking, because similar neurons may be involved in the control of smooth-tracking eye movements in vertebrates.

Neurons which respond selectively to the direction of motion of an object have been found in many animals¹. Objects moving in one direction (the preferred direction) stimulate these neurons maximally, whereas objects moving in the opposite direction (the null direction) inhibit them or leave the resting discharge unchanged. One function proposed for some such movement detectors is to provide a velocity error signal to stabilize the position of the eyes relative to the visual environment^{4–6,11}. It is appropriate to begin with a brief account of this system, for that proposed for tracking is similar in many respects.

Any movement of the eyes will make the image of the environment shift across the retina. If the eyes are to remain fixed relative to the environment, any inadvertent shifts of the retinal image must generate compensatory movements. The only available visual information to control such movements is an error signal indicating the relative velocity between the eye and the environment.

In the classical optomotor experiment an animal is placed inside a rotating striped drum; when the animal starts to follow the stripes, their velocity across the retina (slip speed) decreases progressively as the animal catches up. The slip speed may decrease to very low values, but because it is itself responsible for the turning reflex, it can never reach zero. There is always a steady state velocity error, which is manifest in an increasing position error. For efficient stabilization, the system needs to minimize the steady state error, and so requires a high gain at low velocities. One way to provide this is to have movement detectors which are very responsive to stimuli travelling at low velocities^{5,6}.

Smooth-tracking eye movements are produced when a stationary animal follows a moving object with its eyes. The reflex acts to stabilize the image of the target on the retina. There is good evidence that in man² and monkey³ it is the movement of the image over the retina which activates smooth-tracking; that is, the target slip speed is the error signal in a velocity control system.

When the target is very large, or the background against which it moves is featureless, the situation is comparable with that of the optomotor experiment. But when a small target moves across a normal environment the background introduces complications.

Consider the simple, if idealized, situation of a stationary animal starting to track with its eyes an object moving at a constant speed in a circle around it. As the animal begins to follow the target, the slip speed of the target image decreases, and the image of the background moves in the reverse direction. When the target image is effectively stabilized on the retina, its slip speed approaches zero, while the background now moves across the retina at a rate equal to the eye speed. Any tendency to track small objects moving across a featured environment will be opposed by an optomotor response generated by the background⁷, unless it is inhibited. Moreover, visual neurones concerned in tracking should not interpret the moving background as a target.

A set of visual neurones closely involved in tracking should thus be excited by moving targets but should ignore the background. To do this they must be influenced both by moving targets and by movements of the background. Any directionally selective neurone with a relatively small receptive field could detect moving targets. A directionally selective neurone which responds solely to a moving background needs an extensive visual field and should give a strong discharge only to stimuli which activate a large proportion of the field.

A "tracking" neurone might then combine the properties of these two neurone types in the following way: a "target detecting" neurone with a particular preferred direction would receive an inhibitory input from "background" units with the same preferred direction. When the animal turned to follow a target, the response of "tracking" units with the opposite preferred direction to the direction of target motion would be suppressed, and so would not respond to static features in the environment as the animal moved, whereas "tracking" units responding to the target would be unaffected by the background.

For an animal to stay effectively locked on to a target, the steady state velocity error must be as small as possible. To achieve the necessary high gain in the system, the target detecting neurones should be very responsive to stimuli travelling at low velocities. The background units should be responsive over the range of possible target speeds.

Neurones found in the moth visual system have properties which suggest they are influenced in this way by movements of

objects in the visual field and by movements of the background. Some directionally selective neurones in the superior colliculus of the cat have inhibitory surrounds, which suppress centre responses most strongly when movement through the surround is in the preferred direction⁸.

Single-unit recordings were made in the right anterior optic tract of unanaesthetized adult moths⁹. The anterior optic tract is a large fibre tract which enters the medial protocerebrum at its dorsal anterior margin¹⁰. I shall discuss only one class of neurone recorded in the tract. Evidence will be presented elsewhere that these are output cells from the optic lobe.

Nervous activity was analysed with a special purpose computer (Biomac 1000). The computer was set to count the number of impulses occurring in successive 250 or 1,000 ms intervals, and in the figures the responses are expressed as an average frequency for the integration time.

The moth was placed at the centre of a translucent 'Perspex' hemisphere so that the right eye looked into the bowl. Two projectors were used to throw images on to the back of the dome. One, the movement of which was controlled by feedback, provided oscillating bars, spots or edges; the other presented a continuously moving grating of roughly 30° period, which could cover the whole hemisphere. The left eye could also be stimulated by a continuously moving grating presented through a fibre optic image guide placed 5 mm from the eye, where the image subtended horizontally 110° and vertically 60° of the visual field. The background illumination was 0.02 cd m⁻², the oscillating bars and spots were 0.12 cd m⁻², and the light stripes of the gratings were 0.05 cd m⁻².

The sixty units to be considered here responded only to moving stimuli; they were directionally selective with back to front preferred directions. They responded in roughly the same way to black or white spots, to bars and to edges, both when the leading edge was dark and when it was light. The receptive field centres were plotted on the surface of the hemisphere using a hand-held ophthalmoscope to provide moving 2° spots. The edges of the field centre plotted in this way were ill-defined. The centres were mostly around 30° in diameter, though in some instances they were as large as 90°. They were always situated in the antero-ventral quadrant of the eye. Usually, no more than two units were studied in a single preparation, and it is likely that the same few large fibres were studied on many occasions.

Some of the units gave distinguishable but weak responses to bars moving as slowly as 0.15°/s, others were unresponsive until bar speeds reached 2°/s. All were directionally selective over the whole range of speeds to which they responded (they were tested up to 150°/s). Fig. 1 shows the response of a

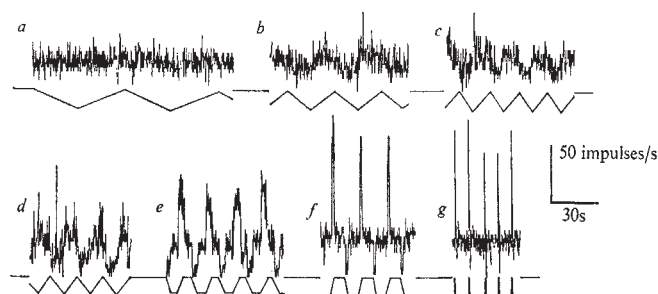


Fig. 1 Response to a bar, 15° high by 5° wide, which swept back and forth in the null-preferred axis. The 25° sweep of the bar lay within the centre field. The bar speeds were: a, 0.8; b, 1.7; c, 2.8; d, 3.6; e, 6.2; f, 20.8; g, 100°/s. The horizontal lines between the traces indicate the zero level of the computer print-out. In this and the following figures, the stimulus monitor below the print-out is a diagrammatic indication of the movements of the bar. Upward excursions signify that the bar moves forwards. Bin width is 250 ms.

typical unit to a light bar moving at different speeds within the centre. The "surrounds" of these neurones probably extend over most of the binocular field⁹. Ipsilateral surround effects were seen most clearly when a grating covering the whole hemisphere, apart from the receptive field centre, was made to move during back and forth sweeps of a bar through the centre. Forwards movement of the surround grating inhibited the response to a bar moving forwards in the preferred direction (Fig. 2*B*) and also slightly inhibited the resting rate. Backwards movement of the surround often enhanced the response (Fig. 2*A*) and induced a discharge when the bar moved very slowly in the preferred direction. With no stimulation at the field centre a surround grating moving in the null direction often suppressed the resting rate (Fig. 2*C*). This inhibition probably arose because the surround grating unintentionally overlapped the centre, the boundaries of which are set somewhat arbitrarily by the plotting technique.

The neurones are probably concerned only with objects that fill a large proportion of their 30° field centres, even

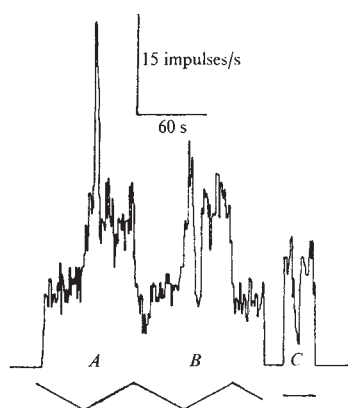


Fig. 2 Response to bar oscillating within the field centre in the null-preferred axis. The bar swept through 20° at 0.4°/s. During sweeps in the preferred direction, the right surround grating moved at 20°/s for a period indicated by the line beneath the print-out. In *A* the grating moved backwards and in *B* it moved forwards. In *C* the bar was stationary and the grating moved backwards. Bin width is 1 s.

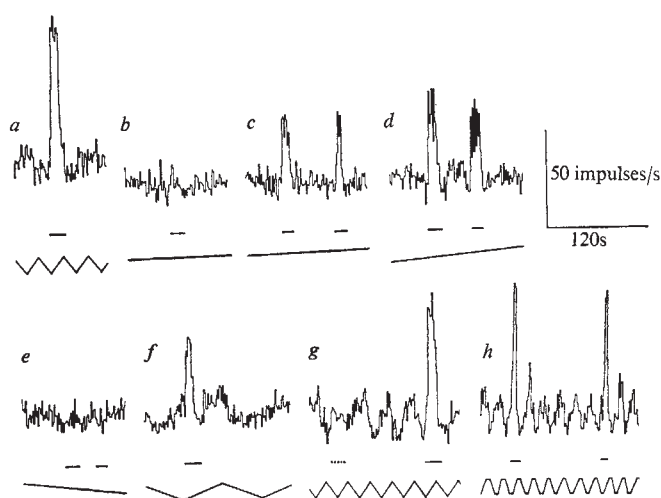


Fig. 3 The effect of left surround grating moving at 16°/s on the response to a bar moving through 25° at different speeds. The solid line beneath the print-out indicates when the grating moved forwards, the dashed line when the grating moved backwards. The vertical position of these lines indicates the zero level of the computer print-out. Traces are arranged in the order in which different bar speeds were tested: *a*, 1.7; *b*, 0.04; *c*, 0.08; *d*, 0.2; *e*, 0.2; *f*, 0.5; *g*, 2.2; *h*, 6.2°/s. In all traces except *e* the grating moved while the bar travelled in the preferred direction. Bin width is 1 s.

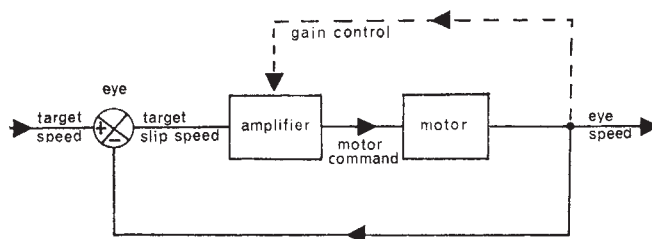


Fig. 4 Block diagram of a possible velocity control system for visual tracking incorporating a gain control. The output of the "tracking" neurones corresponds to the "motor command".

though much smaller stimuli can evoke strong responses. If the surround grating encroached appreciably on to the field centre and moved in the null direction across it, without necessarily overlapping the sweep of the bar, then the response to the bar was strongly suppressed.

Forward motion of a grating presented to the left eye enhanced the response to bar movement in the preferred direction and brought out a response at low speeds (Fig. 3); backward motion of the grating suppressed the response to the bar. The grating had usually no effect on the resting rate.

The facilitatory effects of background movement were most easily examined using gratings presented to the left eye. Fig. 3 shows the effect of a moving grating during sweeps of a bar across the receptive field centre at different speeds. At slow speeds only part of the sweep is shown. When the grating moved forwards, the unit, which was normally unresponsive to the bar moving at slow speeds, gave a strong discharge to bar speeds of 0.08°/s. The amount of facilitation (measured as the increase in firing rate) tended to increase with the speed of the bar.

In some neurones the response to a bar moving at a fixed speed through the field centre was not finely graded according to the speed of the left eye grating. There was no facilitation when the grating moved more slowly than about 3°/s, but for higher speeds (up to 100°/s) the amount of facilitation was remarkably constant. It seems as though the "gain" of the "tracking" neurone is switched according to the direction and speed of the background. In a few neurones the response to a bar moving at a fixed speed through the field did increase slightly as the speed of the left eye grating was raised from about 10 to 80°/s.

Until more is known about the visually guided behaviour of flying moths and about the other neural components of the system little can be said concerning the possible function of the units. The organization of the binocular surround suggests that the neurones respond optimally during turning and so are involved in an orientation response to a moving visual stimulus. The units cannot mediate the tracking of slowly moving targets, because they would respond strongly to slow target slip speeds only when the insect turns faster than 3°/s. But even when the insect fulfils this condition, the slowest slip speed to which the neurones respond (about 0.06°/s) is not impressive. It is, for example, an order of magnitude higher than the slowest slip speed which will induce a crab's eye to follow a moving drum⁴. The neurones might be involved in providing a relatively gross motor command to cope with large velocity error signals; a parallel system capable of more delicate control would be needed to deal with smaller error signals.

In terms of the velocity control system illustrated in Fig. 4, the facilitatory effect of the background will serve to increase the gain when the eye rotates faster than a given angular velocity. Linking the gain to the insect's angular velocity will boost the output of the system for large errors. For example, an additional "back-up" motor may be brought into cope with large errors at certain tracking speeds. Alternatively, these gain adjustments might be used to compensate for non-linearities in the motor system.

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- ¹ Grüsser, O. J., and Grüsser-Cornhels, U., *Ergeb. Physiol. Biol. Chem. Expt. Pharmacol.*, **61**, 178 (1969).
- ² Rashbass, C., *J. Physiol.*, **159**, 326 (1961).
- ³ Fuchs, A. F., *J. Physiol.*, **191**, 609 (1967).
- ⁴ Horridge, G. A., *Symp. Soc. Exp. Biol.*, **20**, 179 (1966).
- ⁵ Collewijn, H., *Vision Res.*, **9**, 117 (1969).
- ⁶ Nuboer, J. F. W., *Pflüger's Arch.*, **315**, 250 (1970).
- ⁷ Rademaker, G. C. J., and ter Braak, J. W. G., *Brain*, **71**, 48 (1948).
- ⁸ Sterling, P., and Wickelgren, B. G., *J. Neurophysiol.*, **32**, 1 (1969).
- ⁹ Collett, T., *J. Neurophysiol.*, **33**, 239 (1970).
- ¹⁰ Strausfeld, N. J., and Blest, A. D., *Phil. Trans. Roy. Soc., B*, **258**, 81 (1970).
- ¹¹ Oyster, C. W., *J. Physiol.*, **199**, 613 (1968).

Sex Differences in Gonadotrophin Concentrations in Infancy

DETERMINATION of serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by radioimmunoassay in groups of prepubertal children of various ages yielded the unexpected result that levels of both gonadotrophins are higher during infancy in girls than in boys^{1,2}.

Serum was obtained from forty-three boys and forty-eight girls (0.3–8.0 yr old) including healthy in-patients and out-patients at the Children's Hospital, and school children. Serum was frozen at -20°C until analysed. FSH and LH were measured by specific radioimmunoassay techniques^{3–5} using duplicate 0.4 ml. samples. Coefficients of variation for these

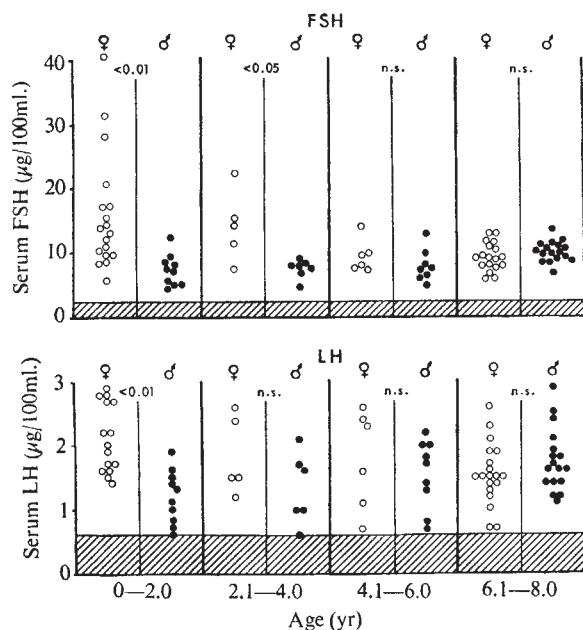


Fig. 1 Individual serum FSH and LH concentrations in boys and girls at 2 yr age intervals expressed as μg LER-907 standard/100 ml. The cross hatching indicates the average minimum level detectable by our assays. *P* values for sex differences are indicated on top of each pair of observations (not significant = $P > 0.1$).

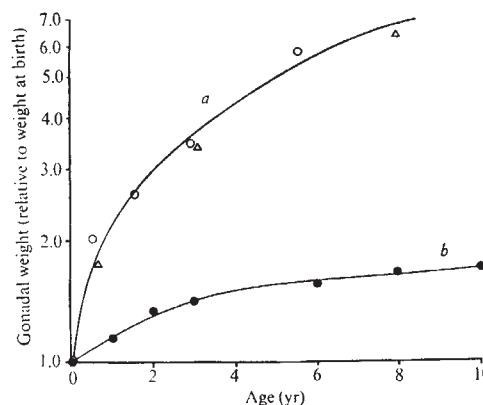


Fig. 2 Semilogarithmic plot of gonadal growth during infancy. Gonadal weight at birth assigned a value of 1.0. Mean weights of gonad pairs at birth were: ovaries 0.31 g, testes ≈ 0.7 g. Values are derived from the literature: $\circ$, Jones⁷; Δ , Mayer⁸; $\bullet$, Schonfeld⁹. *a*, Ovary; *b*, testis.

duplicate determinations were $\pm 4.9\%$ for the FSH assay and $\pm 6.1\%$ for the LH assay.

Individual FSH and LH values plotted by 2 yr age intervals are shown in Fig. 1. Data were analysed by non-parametric statistical techniques— χ^2 for the 0–2 and 6.1–8 yr groups and Wilcoxon two-sample rank test for the other groups. Concentrations of FSH were significantly higher in the 0–2 yr and 2.1–4.0 yr old girls than in boys of similar age. Concentrations of LH were higher in the 0–2 yr old girls. No sex differences in gonadotrophins were observed at the other ages. In girls, concentrations of both FSH and LH diminished significantly from 0–8 yr (FSH: $r = -0.50$, $P < 0.001$; LH: $r = -0.38$, $P < 0.01$); in boys, these concentrations increased slightly during this time (FSH: $r = 0.51$, $P < 0.001$; LH: $r = 0.46$, $P < 0.01$).

We are unaware of any previous reports in which sex differences in gonadotrophin concentrations in infancy were described. Inspection of the data of Lee *et al.*⁶, however, reveals a similar sex difference in concentrations of FSH at 1–2 yr.

Does this sex difference in early infancy have any physiological significance? As Fig. 2 shows, the prepubertal ovary grows more rapidly than the testis, particularly in the first 2 yr of life. It is interesting to speculate that the higher concentrations of gonadotrophin in female infants may be responsible for this accelerated growth. It is not clear whether the increased gonadotrophin concentrations in female infants result from a primary hypothalamic difference or a gonadal feedback difference.

The wide scatter in FSH values in girls younger than 2 yr (Fig. 1) with four of the eighteen values falling in the adult menstrual peak range (20–50 $\mu\text{g}/100$ ml.) is very interesting. Are there cyclic variations in concentrations of hormone at this age and does this explain the wide scatter? Longitudinal studies of individual subjects will be required to answer this question.

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- ¹ Winter, J. S. D., and Faiman, C., *Clin. Res.*, **17**, 646 (1969).
- ² Faiman, C., and Winter, J. S. D., *Proc. Fifty-second Meeting of the Endocrine Society*, 75 (1970).
- ³ Faiman, C., and Ryan, R. J., *J. Clin. Endocrinol.*, **27**, 444 (1967).
- ⁴ Ryan, R. J., and Faiman, C., in *Protein and Polypeptide Hormones*, 129 (Excerpta Medica, Amsterdam, 1968).
- ⁵ Faiman, C., and Ryan, R. J., *Proc. Soc. Exp. Biol. Med.*, **125**, 1130 (1967).
- ⁶ Lee, P. A., Midgley, jun., A. R., and Jaffe, R. B., *J. Clin. Endocrinol.*, **31**, 248 (1970).
- ⁷ Jones, jun., H. W., in *The Biologic Basis of Pediatric Practice* (edit. by Cooke, R. E.), **2**, 1086 (McGraw-Hill, London and New York, 1968).
- ⁸ Mayer, A., in *Biologie und Pathologie des Weibes* (edit. by Seitz, L., and Amreich, A. I.), **1**, 864 (Urban and Schwarzenburg, Vienna, 1952).
- ⁹ Schonfeld, W. A., *Amer. J. Dis. Child.*, **65**, 535 (1943).

Incision of Tissue by Carbon Dioxide Laser

THE radiation produced by a carbon dioxide laser can destroy tissue¹⁻³; exposure to the unfocused beam results in a superficial lesion which develops the characteristics of a burn: erythema, oedema, blistering and charring. If the beam is focused to a spot of 1-2 mm in diameter it incises tissue to a depth of several millimetres, depending on the laser power and focus cone angle and the speed of incision⁴, but no visible damage is caused to adjacent tissues.

For the incision of tissues by burning we would expect the temperature at the point of laser impact to be at least that required for the combustion of tissue. The carbonization of skin occurs between 300° C and 400° C (ref. 5) and a temperature of 530° C causes the combustion of skin⁶. Mullins *et al.*⁷, from optical pyrometer recordings of the laser impact on fire brick, suggested that temperatures of the order of 1,500° C are reached in laser burns. Direct measurement of the temperature of laser-tissue interaction with thermocouples would be of doubtful validity. Existing infrared pyrometers do not afford adequate resolution, and would probably yield misleading results for direct measurement of the temperature of laser-tissue interactions.

In the absence of a direct method of measurement we have examined carbon dioxide laser incisions with high speed close-up colour cine photography in the hope that the colours of the films obtained would indicate the temperature within the incisions.

A carbon dioxide laser of 1.46 m tube length, operating in the TEM₂₀ mode and producing 60 W at 10.6 μ m wavelength,

was used. The beam was directed and focused by a pivoting mirror joint manipulator⁸ which, because of losses at the mirrors, reduced the effective power to 20 W, but focused it to a spot 0.6 mm in diameter (measured experimentally with a silica glass wedge). The power density was thus 1.6 kW cm⁻².

We used 16 mm Eastman Kodak (Magnifax) and Wollensak 'Fastax' cameras at speeds of between 200 and 8,000 p.p.s. The lens system used was a 6 inch 'Raptar' lens with a 9 inch extension giving coverage of approximately 1 cm² and an effective lens aperture of f8. Illumination was provided by two 800 W quartz iodine lamps with 6 inch condensers and extended Fresnel screens. 'Ektachrome' EF 7242 reversal film was used throughout and was rated 125 ASA for exposure on the rat skin and 250 ASA on the liver.

Albino Wistar rats were anaesthetized by 'Halothane' and oxygen, shaved, placed on a small trolley and drawn under the focused laser beam by a variable speed motor. This ensured incisions at a known constant speed.

Films were taken of incisions in the dorsal skin of the rats and, after midline laparotomy, in the anterior surface of the liver. The laser beam was incident normal to the tissue surface and the camera viewed the length of the incision at the point of laser impact, as the animal moved towards the camera.

Further information was obtained from films taken without using illumination, in which the incision would only be visible by light emission from the incandescent combustion of tissue, should this occur in the course of laser incision.

The following points arise from Fig. 1*a* and *b*. (1) At the point of laser impact (*A*) the tissue bubbled as though boiling. This constantly moving surface in the process of destruction glistened but was not incandescent. On films taken with low illumination this area was not visible. This observation is further illustrated by Fig. 2 from a film taken in darkness, which shows a few particles of tissue burning as they pass through the laser beam. It does not show the incision itself and it is clear that the walls of the incision and the principal area of laser impact do not have the incandescent brightness of tissue combustion. (2) The side walls of the incision appear light brown, with vertical ridges of slight charring at intervals. (3) Small particles of dark carbonized tissue (*B*) appear round the edges of the wound but cannot be seen in the depth of the wound. As the incision progressed those at the leading edge moved sideways as the tissue separated and subsequently lay along the edge of the incision. At irregular intervals one of them left the field of view as though blown away (*D*). More frequently a particle burnt at white heat (*C*) and then disappeared as if burned completely. (4) Steam or smoke partly obscured the area of tissue separation and then passed across the field of view. The true pattern of steam dispersal would

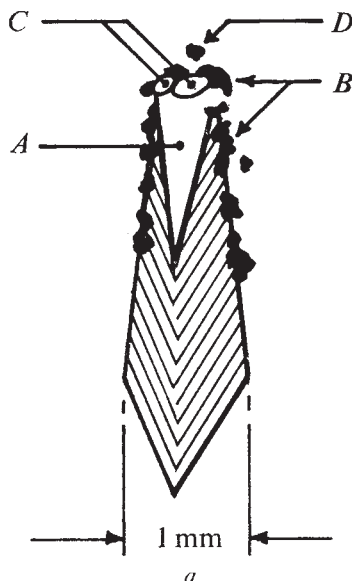
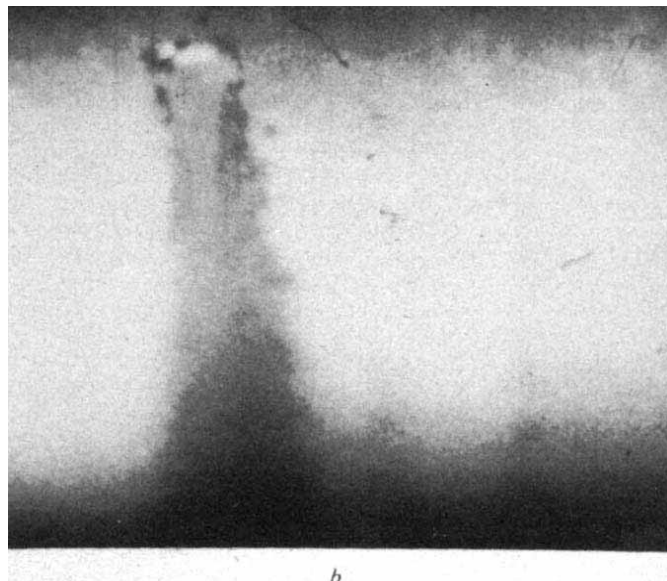


Fig. 1 *a*, Laser incision of rat skin. *A*, Area of laser impact; *B*, carbonized tissue at the margin of wound; *C*, white-hot tissue; *D*, carbonized particle leaving incision. *b*, Black-and-white print from colour cinéfilm at 2,000 p.p.s.



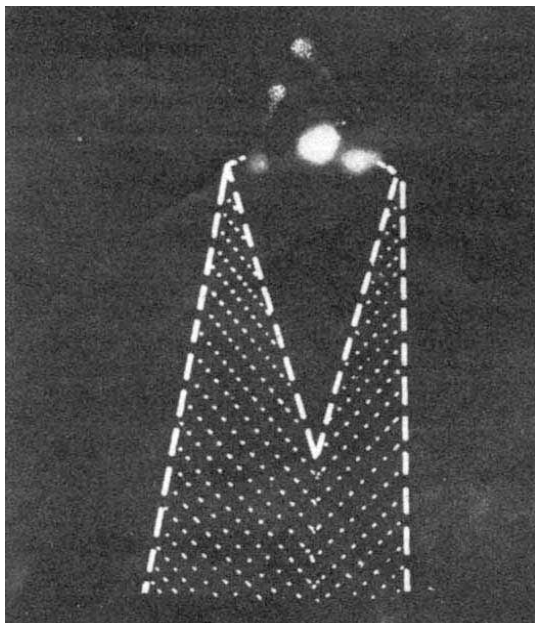


Fig. 2 Laser incision of rat skin without illumination, showing white-hot particles of burning tissue above the incision. The area of laser impact and the walls of incision (marked by dotted lines) are not incandescent (from cinéfilm at 200 p.p.s.).

have been distorted by the air flow from the manipulator and no valid conclusions can be drawn from this. (5) Incisions of the liver are similar to those of skin except that the "boiling" at the focal area is more distinct and there is less charring at the wound margins.

In the conditions of the experiment, a subject temperature of 1,000° C would appear red on the film, 1,500° C orange and 2,000° C bright yellow to white⁹.

The area of laser impact is grey and its temperature is therefore well below red heat (1,000° C). It does not burn and so could not reach the combustion temperature of 530° C. Incandescence occurred only in spots less than 0.2 mm in diameter which existed sporadically above the wound, never continuously round the leading edge nor at the deeper surface of laser action. This emphasizes that the process of incision is not due to tissue combustion. Furthermore, there was no sign of carbonization. Thus, on the basis of colour, the temperature of laser incision must be less than 300° C.

Rat skin contains 67% water by weight (unpublished work of R. R. H.). Steam was seen to be produced and this requires a temperature of 100° C; normal electrolyte concentration raises the boiling point by 0.15° C (ref. 10). The temperature of steam occupying the incision would have been raised further by absorption of energy from the laser beam, but water vapour absorbs little energy at 10.6 μ m (ref. 11) and the temperature would be raised by 0.063° C thereby.

The conversion of water to steam results in a thousand-fold increase in volume. Such an expansion occurring suddenly within cells will undoubtedly cause cell disruption and carry solid cellular material away, causing tissue separation. We suggest that this is the process underlying the "boiling" of tissue seen in the film at the point of laser impact and conclude that incision results from the vaporization of tissue water.

This would mean an incision temperature of 100° C at normal atmospheric pressure plus 0.213° C (0.15+0.063° C). The temperature is unlikely to be higher because the large latent heat of vaporization of water acts as a considerable energy sink. Also, the superheating of cell water before steam formation is improbable at the rate of energy input from the continuous wave laser, and would cool to 100° C on expansion of the steam. These two factors will act as a 100° C thermostat and we conclude that the ablation temperature of tissue when

incised by a carbon dioxide laser is the boiling point of water.

In view of this conclusion how can we account for the marginal carbonization described? Cell debris is carried out of the wound by expanding steam and some is deposited around the edge. As it passes through the beam, it is heated further which will result in its carbonization and explain its presence at the wound margin and not at the area of laser impact. Other particles remain for longer in the beam and absorb sufficient energy to raise their temperatures to ignition threshold. If atmospheric oxygen is accessible they burn, appearing white hot on the film.

The dry pale-brown walls of the incisions are compatible with dehydration without gross disorganization. The macroscopic and microscopic appearance of tissue from the walls is similar to that of identical samples dehydrated by dry heat at 100° C.

Rockwell *et al.*¹² assumed an ablation temperature of 100° C in deriving their heat equation for the speed of laser incision, and our findings confirm their assumption.

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¹ Fine, B. S., Zimmerman, L. E., and Fine, S., *Nerem Record*, 160 (1966).

² Goldman, L., *Biomedical Aspects of the Laser* (Wright, Bristol, 1967).

³ Fox, J. L., *J. Surg. Res.*, 9, 199 (1969).

⁴ Litwin, M. S., Fine, S., Klein, E., and Fine, B. S., *Arch. Surg.*, 98, 219 (1969).

⁵ Moritz, A. R., and Henriques, F., *Amer. J. Pathol.*, 23, 915 (1947).

⁶ Pikkarainen, J., *Acta Physiol. Scand. Suppl.*, 302, 22 (1968).

⁷ Mullins, F., Jennings, B., and McClusky, L., *Amer. Surg.*, 34, 717 (1968).

⁸ Beach, A. D., *J. Sci. Inst.*, 2, series 2, 931 (1969).

⁹ Rieck, G. D., and Berbeek, L. H., *Artificial Light and Photography* (Philips Technical Library, 1952).

¹⁰ Hoyt, C. S., and Fink, C. K., *J. Phys. Chem.*, 41, 453 (1937).

¹¹ McCoy, J. A., Rensch, B. B., and Long, R. K., *Appl. Optics*, 8, 1471 (1969).

¹² Rockwell, R. J., Fidler, J. P., Epstein, R. A., Naprstek, Z., Franke, E. K., Hashimoto, K., and Dreffer, R. L., *Conf. on Lasers in Med. and Biol.*, London (1969).

Metabolism of *Campylobacter* spp. (*Vibrio* spp.) in Connexion with Pathogenicity and the Use of Neuraminidase to suppress Pregnancy

WHILE studying *Campylobacter*, a genus of microaerophilic bacteria including the organism formerly called *Vibrio fetus*, we have obtained some results which may explain, among other things, how these organisms cause infertility in cattle. These results were the outcome of an investigation of the effects of mucin on *Campylobacter*, which we began because, knowing that this organism is often associated with mucous membranes¹, we thought that mucin was probably important for its growth, possibly as a readily utilizable nutrient.

Forty campylobacters from various sources were isolated and cultivated as described before², and five strains representing the various types in the collection were studied in detail. Washed campylobacter cells used oxygen rapidly in a Clark-type oxygen electrode at 37° C (Rank, Bottisham) when mixed with hog gastric mucin (grade II, Sigma) in 0.1 M Sørensen's buffer (pH 7.2). Dialysis showed that low molecular weight substances present in the mucin as supplied were being oxidized. Because this mucin is known to contain some unbound neuraminic acids, and because neuraminic acids may constitute up to 12% of the dry weight of naturally occurring mucus³, we next tested as pure substances the two most commonly occurring neuraminic acids—*N*-acetylneuraminic acid and *N*-glycolylneuraminic acid (Sigma)⁴; in similar conditions the campylobacters oxidized each of these. We therefore tested campylobacters for the ability to inhibit virus haemagglutination and found, contrary to Dennis¹, that chick and human erythrocytes pretreated with culture filtrates were not agglutinated by influenza virus. This property is consistent with possession of a neuraminidase, an enzyme that removes an acylated neuraminic acid from an adjacent sugar residue³. No differences were revealed between the strains, although they were from different sources (strain H22 was from faeces of a healthy pig, strain 16 was from the caecum of a pig suffering from dysentery, strain 271A was from the vaginal mucus of a heifer infected with a strain associated with infertility, strain 187/3 was from faeces of a healthy heifer and strain 102 was from an aborted sheep foetus) and showed differences in other characters.

These findings have at least three implications. First, it may be possible by studying the metabolism by campylobacters of substances associated with cell surfaces to learn how these organisms grow in their natural habitats. They have been regarded as metabolically relatively inert, but our results suggest that they can release and oxidize neuraminic acids. Second, cattle and sheep abortions caused by campylobacters may depend on high concentrations of neuraminic acids in foetal material, in the same way that abortions due to *Brucella abortus* are dependent on erythritol⁵. Third, it is now possible to suggest how *C. fetus* can cause infertility in cattle. Neuraminic acids are components of animal cell surfaces and so may confer specificity important in interactions between cells. *C. fetus* may remove neuraminic acid from the gametes, the zygote or the mucosa of the genital tract, thus changing the surface structure and so preventing either fertilization or successful implantation. Gasic and Gasic⁶ reported that injection of neuraminidase from *Vibrio cholerae* into mice post coitum completely prevented pregnancy, and suggested that study of its effect in primates would be worthwhile. Perhaps *C. fetus* infertility of cattle provides a naturally occurring example of the use of neuraminidase as a contraceptive.

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¹ Dennis, S. M., *Cornell Vet.*, **57**, 630 (1967).

² Morris, J. A., and Park, R. W. A., in *Isolation of Anaerobes* (edit. by Shapton, D. A., and Board, R. G.), 207 (Academic Press, London, 1971).

³ Gottschalk, A., *The Chemistry and Biology of Sialic Acids and Related Substances* (University Press, Cambridge, 1960).

⁴ Svennerholm, L., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **6**, 453 (Academic Press, New York, 1963).

⁵ Smith, H., Williams, A. E., Pearce, J. H., Keppie, J., Harris-Smith, P. W., Fitzgeorge, R. B., and Witt, K., *Nature*, **193**, 47 (1962).

⁶ Gasic, G. J., and Gasic, T. B., *Proc. US Nat. Acad. Sci.*, **67**, 793 (1970).

Buoyancy Regulation in Deep Diving Whales

CLARKE¹ has suggested that the spermaceti of the sperm whale serves as a buoyancy regulator by cooling as the whale dives into seawater of greater density. According to calculations presented¹ a whale of 31,435 kg would have an increased lift of 79.5 kg on a dive from 22.3° C water to the deeper (1,000 m) colder water of 7.8° C. It is further suggested¹ that other cetacea having similar spermaceti organs² may adjust buoyancy in the same way.

To achieve these results it must be assumed that the volume of the whale remains constant during the dive. In experiments with a smaller odontocete *Tursiops truncatus* trained to make controlled voluntary dives in the open ocean we have shown that the shape of the animal changes drastically at depth³. As the dolphin dives down the flexible thorax starts to collapse and at a depth of 300 m thoracic collapse is very marked (Fig. 1).

It seems, however, that thoracic collapse is important primarily in respiratory physiology³. The normal swimming thrust of the animal is such that any out-of-neutral buoyancy values of these small proportions (0.5% of body weight¹) are unlikely to be very significant.

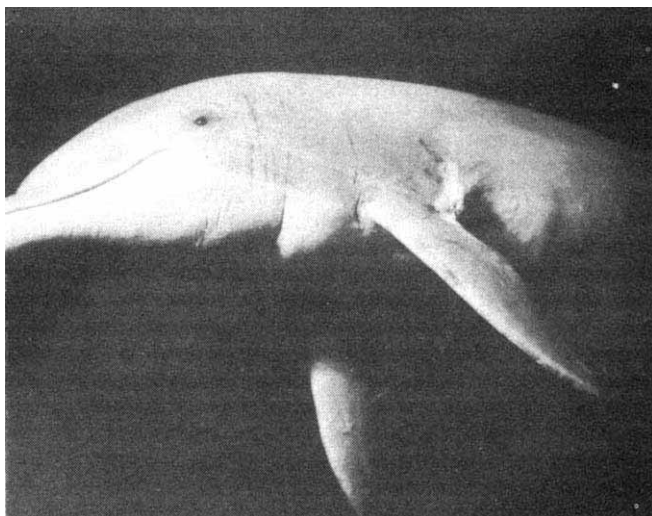


Fig. 1 The dolphin pushes a plunger at the end of a diving test switch at 300 m. This animal had been trained to dive on acoustic command in the open ocean especially for the purpose of studying its diving physiology. The photograph was taken when the dolphin reached the switch and pushed the plunger with its rostrum. (Photograph by courtesy of the US Naval Undersea Center and the Underwater Photographic Unit at Point Mugu.)

A 200 kg dolphin has a respiratory air volume of 10 to 11 l. At a depth of 300 m the compression of respiratory gas would account for approximately 10 kg difference in buoyancy compared with the animal on the surface. If Clarke's¹ sperm whale dives with just 100 l. of air in its lungs (10 to 30% of lung volume¹) the buoyancy difference caused by compression of pulmonary gas, with resultant thoracic collapse, would be greater than could be accounted for by freezing 1,450 kg of spermaceti and much less expensive in terms of energy.

Cooling of peripheral tissues has been demonstrated during dives by other aquatic mammals⁴. No doubt the temperature of at least the more peripheral spermaceti is cooled. Clarke's hypothesis, however, specifies several physiological events not observed in other diving mammals. These include: (1) dilation of the skin blood vessels when the dive is commenced; (2) cessation of bradycardia and vasodilation while the animal is still at depth; (3) passage of water into the nares. The first two specifications appear to be contrary to the oxygen conserving

mechanisms that are primary factors in allowing for prolonged diving^{5,6}.

Many of the deep dives into waters of 7.8° C or colder are feeding forays. The whale is taking food (primarily squid) into its stomach in large quantities. This food is in thermal equilibrium with the seawater and poses an additional heat drain not previously considered.

I thank Mr L. E. McKinley and Professor R. J. Harrison for comments.

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¹ Clarke, M. R., *Nature*, **228**, 873 (1970).

² Lawrence, B., and Schevill, W. E., *Bull. Mus. Comp. Zool. Harv.*, **114**, 104 (1956).

³ Ridgway, S. H., Scronce, B. L., and Kanwisher, J., *Science*, **166**, 1651 (1969).

⁴ Scholander, P. F., Irving, L., and Grinnell, S. W., *J. Cell. Comp. Physiol.*, **19**, 67 (1942).

⁵ Scholander, P. F., *Hvalrad. Skr.*, **22**, 1 (1940).

⁶ Harrison, R. J., and Tomlinson, J. D. W., in *Viewpoints of Biology* (edit. by Carthy, J. D., and Duddington, C. L.), **2**, 115 (Butterworths, London, 1964).

DR CLARKE writes: Dr Ridgway's points will be discussed in detail in an article which will include the observations and calculations on which the original hypothesis rests, but I should like to make a few comments now.

Tursiops is neutrally buoyant at the surface with its lungs full³. Compression would cause negative buoyancy. My observations show that *Physeter* is near neutral buoyancy with its lungs empty. Whether its lungs are full or empty at the start of a dive, it would be near neutral buoyancy at depth (ignoring the temperature effect on water density).

An oil-temperature mechanism for balancing the temperature-dependent water density at depth would be better than an air pressure mechanism which cannot operate in the variable conditions encountered by a sperm whale over its geographical and depth range. The whale would need to be neutrally buoyant at the surface, with the volume of air in its lungs required to balance water density at a particular depth and location.

The oil-temperature mechanism depends on energy which would otherwise be lost as heat, so that it is not "expensive" energetically. Also an air-pressure system has no fail-safe relationship between the length of dive and the energy used. Unpublished observations on sixty-four whales show that if food taken at depth reaches equilibrium with body temperature before the whale surfaces, the volume of food is rarely sufficient to absorb more than about a tenth of the heat required to melt the spermaceti. In one case¹ the volume of food taken in one dive would have absorbed 0.4×10^4 kcalories—a quarter of the heat required to melt the spermaceti. However, the thickness and non-vascularity of the first stomach wall together with vasoconstriction during the dive could delay equilibration until the whale was rising or at the surface, when the cooling effect would be useful.

A delay in the onset of vasoconstriction and bradycardia has been observed in *Callorhinus*² and this would cool the spermaceti at the start of a dive.

Ridgway requires water to enter the nares; I have detected sulphate, indicative of salt water, in both nares of some sperm whales. In reply to his comment on oxygen conservation, this would be affected at the end of a dive if vasodilation included all tissues, but advantages in utilization of heat energy for controlling buoyancy could compensate for this.

The habits of *Tursiops* differ from those of the sperm whale; normally it swims fast, feeds at much shallower depths and its dives are only about one-tenth the duration of those of the sperm whale.

¹ Clarke, R., *Norsk Hvalfangsttid*, No. 10, 589 (1955).

² Irving, L., Peyton, L. J., Bahn, C. H., and Peterson, R. S., *Physiol. Zool.*, **36**, 1 (1963).

Alpha Rhythm in the Blind

THE principal objection to the theory^{1,2} that extra-ocular tremor generates alpha rhythm has been the indisputable fact that it is possible to record it in some patients who have had both eyes removed³.

We have re-examined the eyeless patient described by Shaw, Foley and Blowers³ and find that there are present, across the empty orbits, standing potentials which behave in a manner similar to the normal corneo-retinal potential. When the patient was asked, "Please look to the left and then to the right", an approximately rectangular waveform, of amplitude about 100 μ V peak to peak, appeared between electrodes placed on the inner and outer canthi. This potential was also distributed over the scalp with maximum amplitude anteriorly and minimum amplitude over the occipital region, although it could still be detected there (Fig. 1).

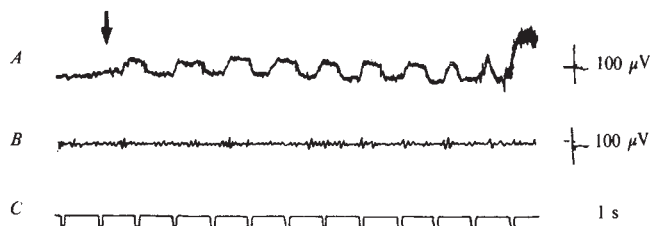


Fig. 1 Sample of EOG record (A) and EEG record (B) from a young diabetic woman of 27 who had both eyes removed because of complications of bilateral glaucoma at the age of 24. This is the patient described by Shaw, Foley and Blowers³. Record (A) is taken from electrodes placed on the inner and outer canthi of the left eye. At the arrow, the request was made, "Look left, look right, look left. . . ." Potential changes 50 to 75 μ V in amplitude (resembling those found in normal subjects due to the corneo-retinal potential) occur concomitant with the commands. Record (B) is taken from leads T₆-O₂ and filtered to select only those frequencies in the range 8 to 12 Hz. There is some degree of correlation between bursts of alpha waves and the excursions in the EOG trace.

The explanation for this phenomenon seems to be that the globe in each orbit has not been removed in its entirety, and that the extra-ocular muscles and a large part of the fundus of the eye remain, including Bruch's membrane which generates the corneo-retinal potential. The magnitude of the potential seemed to be affected only to a small extent, if at all, by alteration in the level of ambient illumination.

Examination of the interior of the orbit showed the lining membrane to be in a state of continual fluttering movement, which was clearly affected by external stimuli such as sounds or light touches on the face. The frequency of many of these movements could well have been within the frequency range of the alpha waves which were concurrently present in the scalp recordings.

It follows, therefore, that the simple, and apparently obvious, fact that an eyeless subject still has alpha waves need not necessarily invalidate the theory that eye muscle tremor produces alpha rhythm.

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¹ Lippold, O. C. J., *Nature*, **226**, 616 (1970).

² Lippold, O. C. J., and Novotny, G. E. K., *Lancet*, **i**, 976 (1970).

³ Shaw, J. C., Foley, J., and Blowers, G. H., *Lancet*, **i**, 1173 (1970).

Period of Adjustment of Rats used for Experimental Studies

MANY workers making pharmacological or behavioural studies allow a period for animals to adjust to their new laboratory conditions but the time they allow is variable and few, if any, reasons are offered for the selection of a particular period^{1,2}.

Our investigations suggest that 4 weeks in a constant environment might be an appropriate period for rats. Hooded Lister rats which had travelled by rail for 5 h were kept in sound proofed rooms at a constant temperature of $23 \pm 1^\circ \text{C}$, with 12 h of light (150 lx) and 12 h of darkness. Twelve females lived in separate cages—two in a room—and could not see each other. Six male litter mates and six female litter mates were also kept in similar conditions but each group of six lived in one cage and each cage was in a room unoccupied by other animals.

The same attendant entered the rooms at the same time each day (approximately 0915 h) and weighed the water bottles; the animals were weighed at this time on every seventh day. Water was freely available, as was Pilsburys pellet diet 41B.

We present here water intake data, which are simple to acquire. Preliminary results also show a decrease in activity, urine and faecal volume, and the same conclusions can be drawn. Fig. 1 shows the fluid intake of a typical female rat. The initial period of acclimatization is followed by a more stable phase showing the 4–5 day variation of the normal oestrous cycle. To obtain a useful rule of thumb quantification of the data, two best-fit straight lines were generated by the method of least squares. The lines, represented by $y = m_1x + c_1$ and $y = m_2x + c_2$, are also shown in Fig. 1. The horizontal distance from the ordinate of the point at which these two lines meet is designated T and acclimatization to the new environment has not occurred before this time has elapsed.

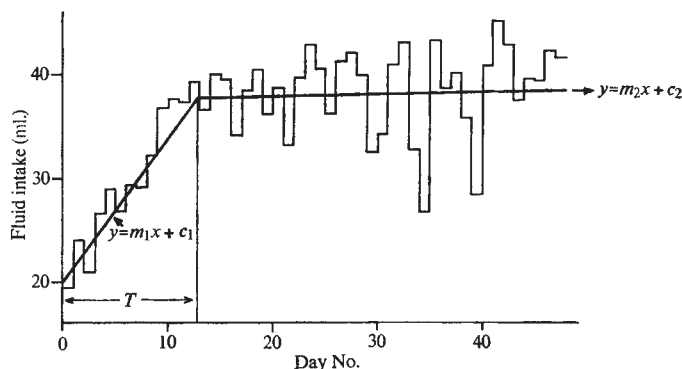


Fig. 1 A typical histogram of fluid intake from a female rat (C118), showing the period of acclimatization and straight lines fitted to the curves.

Table 1 shows the extracted parameters from the studies using separate cages. Although it might seem justifiable to assume that readjustment was adequate a day or so later than T , there must remain some doubt for cases in which m_2 is clearly significant. The acclimatization effect for rat C115 could not have been safely neglected in much less than 28 days.

Table 2 compares the mean parameters for the animals in separate cages with the mean values for the groups. This demonstrates that isolation is not an important factor in either sex.

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¹ Perhach, jun., J. L., and Barry, H., *Physiol. Behav.*, **5**, 443 (1970).

² Stern, J. J., *Physiol. Behav.*, **5**, 1277 (1970).

Table 1 Extracted Parameters of Fluid Intake for Female Rats in Separate Cages

Rat No.	Initial age (days)	m_1 (ml. day ⁻¹)	c_1 (ml.)	m_2 (ml. day ⁻¹)	c_2 (ml.)	T (days)
C108	24	2.1	10.1	0.8	22.5	7
C109	24	2.0	9.5	0.2	29.3	11
C110	24	1.0	18.4	-0.2	33.4	14
C111	24	2.1	9.1	0.9	24.0	9
C112	24	1.1	14.8	0.5	29.5	17
C113	24	1.4	15.2	0.3	32.7	14
C114	32	0.9	27.5	0.2	45.3	22
C115	32	1.0	19.2	-0.4	46.3	25
C116	32	1.5	20.3	0.2	33.6	10
C117	32	1.2	21.2	-0.1	41.0	16
C118	32	1.4	19.9	0.1	37.6	13
C119	32	0.7	27.0	-0.1	38.9	17

Significance of Sexual Dimorphism of the Anal Fin of *Polypteridae*

THE anal fin of the female of both *Polypterus* and *Calamoichthys* is narrow and pointed, whereas that of the male is wider and rounded, and can be erected into a cup-shaped organ. This difference was described for *P. bichir* by Leydig¹, who suggested that internal fertilization occurred, and subsequently Harrington² stated that the anal fin of the male "is undoubtedly a copulatory organ". Budgett³, noting sexual dimorphism of the

Table 2 Ages, Sexes and Mean Values of Extracted Parameters for Groups of Six Rats living Separately or Together

Rats	Sex	Initial age (days)	m_1 (ml. day ⁻¹)	c_1 (ml.)	m_2 (ml. day ⁻¹)	c_2 (ml.)	T (days)	
C108–C113	Female	24	1.6 (0.5)	12.9 (3.8)	0.4 (0.4)	28.6 (4.5)	12 (3.7)	In separate cages
C114–C119	Female	32	1.1 (0.3)	22.5 (3.7)	-0.02 (0.2)	40.5 (4.8)	17 (5.6)	
Group	Female	28	0.9	11.5	0.03	27.0	19	Living in groups
Group	Male	28	0.9	13.9	0.2	31.6	23	

Figures in brackets are standard deviations.

Table 1 Size of Testes Taken from *Polypterus*

Fish	Total length (mm)	Total weight (g)	Right testis		Left testis		Total testes weight (g)
			Length (mm)	Maximum width (mm)	Length (mm)	Maximum width (mm)	
<i>P. bichir</i>							
	515	880	21	7	29	5	0.67
	434	435	42	5	43	4	0.44
<i>P. endlicheri</i>							
	450	550	15	6	20	6	0.39
	437	515	23	5	20	4	0.29
	456	690	21	6	15	5	0.36
	414	415	20	7	16	6	0.45
	392	360	42	3	30	2	0.23
	428	450	22	4	30	4	0.24
<i>P. senegalus</i>							
	247	68	60	2	45	3	0.09

anal fins of *P. senegalus* and *P. bichir* (= *P. lapradei*), wrote that "it remains to be seen to what use the male *Polypterus* puts this modified anal fin", while he dismissed Leydig's suggestion that fertilization is internal. Traquair⁴ described sexual dimorphism of the anal fin for *C. calabaricus* but did not comment on the possible function of the anal fin of the male. Recent work by Arnould^{5,6}, who described the spawning behaviour of *P. senegalus* in aquaria, shows that the anal fin of the male undoubtedly forms a copulatory chamber. During spawning the male swims alongside the female with its anal fin folded up like a gutter towards the vent of the female, fertilizing the eggs as they are laid.

None of these authors, nor Daget *et al.*⁷, however, makes any suggestion as to why fertilization should need to occur in a copulatory chamber, but the reason might well lie in the gross disparity in size of the male and female gonads. The fully developed female gonads always completely fill the body cavity, which is very large in members of the *Polypteridae*, whereas male gonads never develop further than thread-like bodies, with a few flattened lobes at intervals along their length. Table 1 gives dimensions of testes collected from *Polypterus* caught in the River Niger at Shaguna, Nigeria, in August and September 1965 at a time when female fish had either fully developed gonads or were spent. I have never seen, either on this occasion, or during previous work on the fishes of the Niger system, a male *Polypterus* with large, swollen testes from which running milt could be obtained by sectioning, as is common with most species of fish. The testes of *C. calabaricus* are also very small, being described by Traquair⁴ as "very minute . . . each being a small oval body $\frac{3}{16}$ inch (5 mm) in length in a male of 10 inches (254 mm)", although he did not state at what season he made this observation. According to Budgett⁸ the sperm too are minute, those of *P. senegalus* and *P. bichir* being one-sixth the diameter of a red blood corpuscle (species unrecorded). It seems almost certain that the function of the anal fin of the male is to conserve the small amount of milt which is produced. A similar function has been ascribed by Iles⁹ to the anal fin of male *Mormyrus kannume*, in which the fully developed testes are also small.

Polypterus show a pre-spawning display which, in the case of *P. senegalus* kept in aquaria by Arnould⁶, lasted 6 weeks. Although this display is unlikely to be as prolonged in natural conditions, it seems to be essential for ovulation. Both Budgett⁸ and Harrington² noted that at most only a few free eggs ever

occurred in the body of female *Polypterus*, and I have never caught a female from which eggs could be extruded by gentle pressure, the condition usually known as "running ripe". It seems very probable that this display ensures controlled liberation of the ova, this being essential because the total volume of the eggs is much greater than that of the copulatory chamber, which has a volume of approximately 5 ml. in *P. bichir*, compared with a total egg volume of 245 ml. in one female examined; this fish measured 582 mm total length and weighed 1,250 g and its gonad weighed 118 g and contained $39,760 \pm 2,600$ eggs (95% confidence limits). (In *P. senegalus* the volume of the chamber is approximately 1 ml., and in *P. endlicheri* 10 ml.) It is possible that the eggs are shed singly, because Arnould⁶ observed isolated eggs laid on aquatic plants.

The necessity for display to precede ovulation would also appear to explain the long history of unsuccessful attempts to obtain artificial fertilization of *Polypterus*. Both Harrington² and Arnould^{5,6} attempted it, and it is a matter of zoological history that Budgett made it a major objective of his life's work, but with only one success¹⁰.

It is interesting that this "primitive" family, in evolutionary terms, has a complex reproductive mechanism.

The observations represented in this paper were made while I was a member of the First Kainji Biological Research Team. Their fragmentary nature is due to the significance of the small size of the testes not being realized until the spawning season was almost complete, and females with full gonads almost unobtainable.

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¹ Leydig, F., *Z. Wiss. Zool.*, **5**, 40 (1853).

² Harrington, N. R., *Amer. Naturalist*, **33**, 721 (1899).

³ Budgett, J. S., *Trans. Zool. Soc.*, **15**, 323 (1901).

⁴ Traquair, R. H., *Proc. Roy. Soc. Edinburgh*, **5**, 657 (1866).

⁵ Arnould, J., *Bull. Mus. Hist. Nat., Paris*, **33**, 391 (1961).

⁶ Arnould, J., *Acta Zool.*, Stockholm, **65**, 191 (1964).

⁷ Daget, J., Bauchot, R., and Arnould, J., *Acta Zool.*, Stockholm, **46**, 297 (1965).

⁸ Budgett, J. S., *Proc. Camb. Phil. Soc. Biol. Sci.*, **10**, 236 (1899).

⁹ Iles, R. B., *Nature*, **188**, 516 (1960).

¹⁰ Kerr, J. G., *The Budgett Memorial Volume* (Cambridge University Press, 1907).

BOOK REVIEWS

Medicine in Society

The Doctor and the Social Services. By Huws R. Jones. (University of London—Heath Clark Lectures 1969.) Pp. 57. (Athlone: London, April 1971.) £0.90.

In these three lectures Mr Huws Jones calls attention to the need for better understanding by medical men of available social services for the care of the sick and disabled. He complains somewhat of medical lack of knowledge and even of sympathy with the aims of these services. Social workers can contribute through their awareness of local authority responsibilities in coping with bad housing, and they can also help to abate the effects of poverty and ignorance. Even therapeutic achievement which saves lives can add to the numbers of handicapped children and of the aged and infirm in their homes. In former years the tuberculous were removed to sanatoria to reduce the risk of transfer of infection to the remaining members of the family. We have now triumphed over tuberculosis. On the other hand, successes in the drug management of the mentally disturbed are returning home many somewhat embarrassing problems, whose care can impair the unity and even the economic viability of the family. Pressures may once again develop for an expansion of mental institutional care. Workers in social psychiatry may be splendid in helping but they cannot replace informed medical judgment.

Mr Huws Jones suggests that doctors must become more communicative with their lay helpers in relation to the problems of their patients. Doctors are already working with non-medical scientists in the hospitals, but the latter are only concerned with one bit of the total problem. Surely the patient's individual interests are paramount and a policy of confidential trust can often be of the greatest help to the sick man's hope of wellbeing. Already there is much public fear and anxiety about computerized record linkage revealing private matters.

Suggestions for unification and re-organized management of the various streams of medical service and collateral

social services are already being drawn up, but administrative change alone will not make the service either less costly or more efficient. Care of the patient at the professional and technical level is still the basis of the doctor's contribution to human welfare.

The recommendation in the Todd Report of early substantial additional teaching on behavioural and social sciences dilutes the primary object of training the doctor, which is to familiarize him with the biological and medical sciences which will enable him to be a competent judge of human physical and functional illnesses. It is only in his later clinical work with various classes and conditions of men that he becomes aware of the importance of the general social and economic background, and when faced with this challenge he appreciates the necessity for further study in the social sciences. Only when he is a good judge of the course and consequences of illness can he forecast their effect on the family, and in turn the family will impose on him their trust and confidence as they seek advice and help. In dealing with this burden, however, he must use available services about which he can readily learn at a later stage of the curriculum and early in his postgraduate training.

The competent doctor will always have a great contribution to make in the elimination of sickness. General practitioners are already reorganizing themselves for a more efficient contribution to the communities which they serve and without their guidance, advice and support much well meaning kindness could do but little. A sick society needs sociologists, but the individuals need doctors.

J. MCMICHAEL

Government Information

The Central Office of Information. By Sir Fife Clark. (The New Whitehall Series, No. 15.) Pp. xii+184. (Allen and Unwin: London, February 1971.) £2.50.

THE basis of a sound democracy is an informed public. The work of the Government must be made known to

that public if it is to be carried out effectively. Among the information services of the British Government, the Central Office of Information (COI) is the most important, and is the subject of this, the fifteenth book in the "New Whitehall Series", commissioned by the Royal Institute of Public Administration.

Reading this book should convince the layman that there is considerably more information from an official source than he might otherwise have imagined. It is the COI which arranges the professionally prepared material on a variety of subjects from decimalization to improvement grants for home ownership.

The bulk of the work of the COI, however, is aimed overseas and information about Britain, its policies, achievements and traditions is disseminated together with books of an educational nature at a price acceptable to students in underdeveloped areas. The office uses all the skills of the trained communicator, as a large proportion of its staff are professionals recruited from the world of television, journalism, films and design, a worthy quality in the Civil Service, which has suffered many accusations of amateurism.

In addition to the detailed description of the history, constitution and function of the COI the most stimulating feature of the study is Sir Fife Clark's examination of the means whereby the information services are shielded from possible governmental pressure to misuse their influence. The COI was born in a spirit of suspicion and has spent its early years developing safeguards to win around the suspicious. The author has been its director general for fifteen years and it is his contention that accusations of partiality are avoided in the following ways: the COI does not write or edit ministerial notices, but merely distributes them; it does not advertise during general election periods and no money is spent on publicity for legislation still in progress.

The political controversy concerning government information services has largely died down and it is now generally

acknowledged that government publicity, particularly for distribution abroad, is essential.

D. E. WOODHAM

Plant Chromosomes

Chromosomal Evolution in Higher Plants. By Ledyard G. Stebbins. Pp. viii+216. (Arnold: London, March 1971.) £4 boards; £2 paper.

FOR a generation now of biologists, or more precisely botanists, the meaning of cytology has narrowed almost exclusively to the description of the condensations and movements of chromosomes in cell division. We have for many years taken it for granted that neither the evolution nor the ecology nor the taxonomy of plants can properly be discussed without relating them to the karyotypes. Consequently, and quite rightly, many botanists regard the handling of chromosomes as part of their stock in trade and for many it is very nearly their only pursuit, the only path to an understanding of the biology of plant populations. We owe this attitude to a very few who through their writings have come to be the interpreters of chromosome shapes and numbers; who, starting from the simple notion of the chromosome theory of heredity, have established the principles by which the chromosomes determine, or at any rate mark, the pathways of ecological adaptation and evolution. Professor Stebbins is one of them, and any book by him carries the print of authority.

This book is one in the Contemporary Biology series edited by E. J. W. Barrington and A. J. Willis and it is a shortish textbook aimed, I guess, at the first or second year student, although it must also become a useful handbook for more advanced students. Its style is straightforward and its contents are simple and arranged in a logical progression of ideas, starting with the function and organization of chromosomes and progressing through descriptions of the origin of variation in chromosome shape, size and number to the ecological and geographical consequences of karyotype variation. The book is plentifully illustrated, mostly with well chosen and well designed diagrams or clear drawings of chromosomes and karyotypes which are very easy to understand, and with maps and one or two photographs. There are also several informative tables.

A few minor criticisms can be made. Occasionally a technical term slips in which is not explained in the text or mentioned in the index. The discussion of heterochromatin I found interesting, but over-simplified. It left me with the impression that it is a material the function and properties of which can be described in a particular way, whereas it is, in fact, a term which can have almost as

many meanings as there are people who use it. The rather narrow emphasis leads to some confusion later, when heterochromatin is treated as a disposable component of chromosomes to account (in the accepted manner) for changes in basic number. Rather more serious is a degree of confusion which arises from the author's efforts to treat the evidence for the correlation of karyotype evolution (asymmetry, for example) with ecological adaptation in a wholly critical manner. Inevitably, one is never certain whether the author wishes one to believe that morphological and ecological specialization results in or from asymmetry: whether it is causal, consequential, conditional or coincidental.

With the chapters on polyploidy, however, the author does very well. The treatment is lucid and he sweeps away, thank goodness, much of the rigid and stereotyped thinking on the subject that has been almost axiomatic for many years. It is quite clear from this section what role polyploidy has played in the generation of variants and the conservation of variation, in the spread and adaptation of populations. One derives from this account a clear impression of how plants evolve, and an insight into the complexity of the organization of plant populations as adapting breeding groups. Although Professor Stebbins sticks meticulously to the taxonomists' nomenclature the conviction must steal over any reader that, for example, the arbitrary notion of the species or genus as a definable level of biological organization is misleading and irrelevant, and that the key to understanding plants is not in classifying and naming them but in analysing their breeding systems. I have read more inspiring books on chromosomes, and more comprehensible ones, but there are not many which could give the non-specialist and biology student a neater account of chromosome evolution.

B. S. COX

Full Colour Pathology

A Colour Atlas of General Pathology. By G. Austin Gresham. (Wolfe Medical Atlases—2.) Pp. 365. (Wolfe Medical: London, May 1971.) £3.75.

WHETHER the student beginning to study general pathology should use an atlas, an illustrated textbook or both is disputable. Teachers tend to favour textbooks, while students often like atlases and seem to benefit from them if they are relevant, and the pictures show clearly what they are meant to show and are accompanied by captions or explanatory sections. A justification of the atlas as a teaching aid is that students, while understanding much of what they read, are apt to have difficulty in identifying and interpreting what

they see under the microscope.

The atlas produced by Dr Gresham is a laboratory manual designed to supplement rather than replace the textbook. There are 435 colour photographs (chiefly 8×5 cm) with short explanatory captions on the facing page, and each group of between two and twenty or more pictures is preceded by a few paragraphs outlining the subject and introducing the student to the principal concepts of histopathology. The first section of the atlas is devoted to normal cells and tissues and includes brief discussion of electron microscopy, histochemistry, fluorescence microscopy and autoradiography. Then follow sections on variations in cell growth and differentiation, and on cell damage. The greater part of the book deals with responses to injury, including inflammation and repair, and with patterns of disease including infection, trauma, vascular disturbances, allergy, intoxications, nutritional disturbances and neoplasia. There is a short set of pictures illustrating artefacts and the atlas ends with a glossary, list of further reading and index. It thus covers the topics usually dealt with in a course of general pathology.

The success of a book of this kind obviously depends very largely on the quality of the pictures, and most of these are good. There are several lapses, however, such as tissues stained with haematoxylin and eosin appearing dark blue-green (Figs. 151 and 152) or bright yellow (Figs. 396 and 416), emphasizing the fact that reproduction of photomicrographs still presents considerable problems. Some of the details described in the captions are difficult to see and a few arrows would help. The mixing of macroscopic pictures and photomicrographs adds interest to the illustrations.

The text is simple and direct, with a tendency to a vernacular style, and controversial statements are few. A future edition might benefit from an explanation of teratomas (illustrated but not discussed) and embryonal tumours, as well as a section on carcinogenesis illustrated with animal material. The number of printing errors is regrettably large. To give examples, the magnification of Fig. 237 is surely wrong, Figs. 388 and 389 are reversed, the heading to page 330 is truncated and the main index heading of Copper is omitted. There are irritating errors of punctuation on pages 247 and 281. "Lipoid" is used instead of the more usual "lipid" throughout.

I believe, however, that many students will like this book and benefit from it. The price is reasonable, especially taking the large number of colour pictures into account. The second of a series of atlases, it is to be followed, according to

a list in the front of the book, by volumes on twenty-four further subjects.

P. J. SCHEUER

Peptides

The Organic Chemistry of Peptides. By Harry D. Law. Pp. viii+235. (Wiley Interscience: London and New York, December 1970.) £4.25.

ONE of the attractions of peptide research lies in the varied chemistry which it involves; whereas the name centres attention on the backbone of the molecule, the physical, chemical and biological properties depend largely on the nature and position of the side chains—alkyl, benzenoid, and heterocyclic—with a selection of simple functional groups. An immense amount of work has been devoted to the perfection of the organic reactions used in the analysis, structural investigation, and synthesis of peptides, and not all the results of this research have been assimilated into the body of general organic chemistry. This book is therefore most welcome and timely. It reviews methods for the determination of the primary structure of peptides and methods of synthesis, discussing the underlying chemistry; naturally occurring peptides (in which are included insulin and ribonuclease, and those with structural features not typical of proteins) and their synthesis are surveyed; there is a valuable chapter on structure and biological function, which rightly includes proteins in its scope and outlines proposed mechanisms of enzyme action; and the volume ends with an account of the biosynthesis of proteins.

An interesting feature is the incorporation in the text of exercises designed to make the reader think ahead by presenting him with the problem which is to be answered in the following section. This is an approach which deserves careful evaluation and consideration for more general use. Other exercises are of the more usual kind, not specifically dealt with in the text but relevant to material already discussed, and answers are appended.

I noted very few errors but comment is needed on one point. In the discussion (page 84 *et seq.*) of active esters reference is made to the relationship between the pK_a of the phenol and the rate of aminolysis of the ester derived from it, and the data of Pless and Boissonnas are quoted; these figures refer to solutions in aqueous dioxan. It is unfortunate that in the subsequent passages these same pK_a values (about two units higher than those for aqueous solutions) are used without qualification, and although the answer to exercise 3.16 (page 214) refers to this point, it seems too much to hope that the reader should not be confused to find other values quoted elsewhere.

The text is intended for use in honours degree courses at the end of the penultimate year or early in the final year. It is perhaps questionable whether, if a broad grounding in chemistry is to be given, the time required can really be afforded. The author has shown convincingly that important chemical concepts can be exemplified very suitably in peptide chemistry, but of course it does not necessarily follow that these are the most appropriate examples. To me it seems probable that this volume will be found most useful in graduate studies; indeed, its price is likely to put it out of the reach of most undergraduates, unless a paperback edition is offered. As a text for graduates and particularly for those starting research in this field its value would be greatly enhanced by the inclusion of references to the original literature.

G. T. YOUNG

Science of the Sea

The Science of the Sea: a History of Oceanography. Edited by C. P. Idyll. Pp. viii+280. (Nelson: London, 1971.) £6.30.

THIS book, the latest of a number of popular books dealing with the oceans which have appeared in recent years, covers a wide range of topics, going beyond the science of oceanography to include a variety of applications. Its subtitle is a valid description in the sense that the historical development of each topic is used to introduce an account of its present status. In most cases an assessment of prospects for the future is also given.

In the preface, the history of oceanography as a science is said to have begun on January 3, 1873, when HMS Challenger made the first soundings and dredge hauls of its famous voyage. This definition has not, however, inhibited the authors from going much further back in tracing early work, even to the voyage of Queen Hatshepsut of Egypt to the "Land of Punt" in about 1500 BC. One author cites the view that Aristotle was the father of marine biology and another that chemical oceanography was born when Robert Boyle published a well known paper in 1670. In a date list at the end of the book, Edward Forbes and Matthew Fontaine Maury are credited as the "founding fathers of oceanography".

Of the ten chapters in the book, each by an expert in his field, the first five are concerned with the more basic scientific aspects and cover the physics, chemistry and biology of the sea and the topography and geology of the seabed. Two of the following chapters are devoted to fisheries and fish farming and another to mineral resources and power. There is a chapter on underwater archaeology, which starts with a brief history of

diving techniques, and the final chapter deals with submarines and other underwater vehicles and the attempts which have been made to set up seabed habitats.

In a collective work of this kind there is always the danger of repetition and unevenness of style. On the whole these dangers have been avoided and the nine authors who have contributed to the book have achieved a coherent treatment. Much of their subject matter will be familiar to students of oceanography and to people whose general scientific interests have kept them in touch with marine topics. Such readers will probably find more interest in the ideas which the various contributors express about likely developments in the future. Some make rather cautious and sober assessments, as in the chapters on food resources of the sea and the prospects for fish farming. The latter has great possibilities for expansion but there are also real problems to be overcome. The writer makes the point that these are socio-economic rather than biological, though perhaps the more difficult for that. The author of the final chapter, referring to natural resources of the sea in more general terms, allows himself a more sanguine view which ventures into the realm of science fiction.

The book is well illustrated throughout with photographs and drawings. In addition there are four "colour essays", inserted at appropriate places in the text, each consisting of eight pages of coloured illustrations prefaced by a short introduction. The chief value of the book is probably as a layman's guide to the oceans: what we know about them today, how this knowledge has been acquired and what developments may be expected in the future. In taking such a broad view it cuts across the boundaries of geography, history, science and technology but it is none the worse for that.

K. F. BOWDEN

Many Particle Systems

Quantum Theory of Many-Particle Systems. By Alexander L. Fetter and John Dirk Walecka. (International Series in Pure and Applied Physics.) Pp. xiv+601. (McGraw-Hill: New York and Maidenhead, March 1971.) £9.35.

DURING the past twenty years, the fundamental theory of many body systems has been developed into an imposing and solid edifice. Although various aspects of this theory appear in several books and monographs, no really thorough and satisfactory text has been available for the use of students proposing to study, and ultimately to work, in this field. This book has been written to remedy this situation by two university teachers

experienced in introducing many particle theory to graduate students at Stanford University. Professors Fetter and Walecka are well known for their distinguished researches into the theory of quantum fluids and nuclear physics and there can be few scholars as well qualified to undertake this task.

Several approaches to the theory of many particle systems are possible, but in this book the authors have chosen to present a unified account from the viewpoint of non-relativistic field theory. Following an introduction to the basic ideas of second quantization, the reader is led into a discussion of the powerful Green's function techniques, as well as to the Feynman-Dyson perturbation theory and the diagrammatic method initiated by Goldstone. These formalisms are first applied to the determination of the properties of the ground and low lying excited states of a system, and subsequently the theory is developed so that it can be applied to systems at a finite temperature. In a further section, the method of canonical transformations is introduced in connexion with a discussion of both interacting Bose and interacting Fermi gases. Throughout, techniques are illustrated with reference to the electron gas, including descriptions of collective modes of oscillation in plasmas, and to the hard sphere Fermi and Bose gases. To follow the basic material, it is necessary to be acquainted with some parts of statistical mechanics and the necessary discussion of systems with variable numbers of particles, based on the grand canonical ensemble is given in a separate chapter.

The basic theory of many particle systems has received a multitude of applications in the varied fields of nuclear, low temperature, solid state and molecular physics, and while no single book would be expected to cover each of these fields in depth, a remarkably successful attempt has been made to show in the second half of the book the unity of theory underlying these topics. The five physical systems selected for review in separate chapters are nuclear matter, interacting electrons and phonons, superconductors, superfluids and finite nuclei. The treatment of each of these topics is self-contained, each chapter starting with a short review of the experimental and theoretical situation, which provides necessary background for a newcomer to the field.

Throughout the book the level of treatment is that appropriate to an American graduate course. It could be followed by a British student in his first postgraduate year, who had acquired a good knowledge of quantum mechanics in his undergraduate career, together with some familiarity of thermodynamics, statistical mechanics, electromagnetism and mathematical methods.

Plenty of examples, some of considerable interest and of varying difficulty, are given at the end of each chapter, enabling the reader to test his skill and mastery of the material.

This is clearly a very useful and important book that can be warmly recommended to research students beginning work in any of the fields based on many particle theory. The material has been well selected and the treatment is always clear and accurate. A word is in order about the physical appearance of the book, which is of such importance in a work containing a high percentage of mathematical formulae and in this respect the high quality and legibility expected from a volume in the International Series in Pure and Applied Physics is, in general, successfully maintained, although wider margins would have removed a somewhat crowded appearance on the printed page.

B. H. BRANSDEN

Helium-4

Helium-4. By Z. M. Galasiewicz. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Selected Readings in Physics.) Pp. vii+338. (Pergamon: Oxford and New York, April 1971.) £3; \$9.50.

THIS book comprises an introductory essay of sixty-odd pages on helium-4, from its discovery in 1868 in the Sun's atmosphere, to recent ideas about the exact nature of its superfluid phase, plus an extensive collection of relevant papers, both experimental and theoretical, which supplement the author's introduction. The latter is a very readable account of the historical development of the various concepts that are currently in use in the field of superfluid helium compiled by one who has had the privilege of studying for some time under Professor N. Bogoliubov in the USSR. As such, reading this text and familiarizing oneself with the contents of the reprinted papers would serve as an excellent introduction to this field of physics for prospective newcomers whom I would expect to comprise principally of low temperature research students just embarking on their studies. More experienced workers, who learned their subject mostly from review articles, would, I have no doubt, find the author's insight into the historical development of the subject both entertaining and enlightening.

This book is concerned principally with phenomena in bulk liquid helium. The reprinted papers are classified into an experimental section comprising twelve papers and a theoretical section comprising seven papers. Most of the papers chosen not only illustrate the

author's introduction but are at the same time acknowledged key papers in the development of the subject. This is especially true of the experimental papers although there are some notable omissions such as the pioneer papers on quantization of circulation, the determination of the phonon excitation curve in bulk helium by neutron scattering, the analogue of the Josephson effect in superfluid helium and possibly others. On the theoretical side the reprinted papers are all older than fifteen years and were acknowledged milestones in their day although the ideas presented in some are not now considered correct. For example, Landau's well known explanation of superfluid flow in terms of the phonon excitation spectrum is not widely believed today, one reason being that his explanation depends on the phonons being exact excitations of the superfluid system. Once we realize that the excitation curve is in reality a plot of that ω , for which the Van Hove $S(q, \omega)$ is a maximum, versus q then it is seen that no absolute meaning can be attached with drawing a tangent to the curve passing through the origin to determine a critical velocity. Feynman and Pitaevski's works on the excitation spectrum are included but the relevance of these papers to the property of superfluidity is only tenuous if one refutes the validity of the Landau argument. Nowadays the feeling seems to be that a proper explanation of superfluidity should result along the lines of some macroscopic coherence, like in the BCS theory of superconductivity, establishing itself in the helium system below the λ -point. The exact nature of this coherence is still a matter of controversy today, some believing that superfluidity is somehow essentially connected with coherence resulting from the existence of a single particle condensate while others would argue that it results from a Bose analogue to the pair condensate of superconductivity, and yet others would claim it to be a combination of both. Professor Galasiewicz, influenced as he is (see preface to *Superconductivity and Quantum Fluids* by Z. Galasiewicz, Pergamon (1970)) by Bogoliubov's ideas and methods, is a supporter of the single particle condensate explanation but he has chosen not to include any of the many recent theoretical papers on the theory of superfluidity in this book and contents himself with just reprinting Bogoliubov's original paper. Until a consensus of opinion, which clearly favours one recent model over all the others, emerges, I think this is a wise decision in a text of this nature.

This book is hard bound and the printing quality is very good. Its cost seems a trifle high, but, in spite of this, I have no hesitation in recommending it.

W. A. B. EVANS

CORRESPONDENCE

Research Reorganization

SIR,—I would like to refer to your editorial entitled "Keeping a Straight Bat" (*Nature*, 231, 2; 1971) in which you refer to the various courses of action which are open to Mrs Thatcher, Minister for Education and Science, with respect to the support of research. You say, "Thus it may be possible enormously to increase the amount of research carried on within the academic section by lumping together those activities of the research councils—the MRC and the ARC as well as the Science Research Council—which are concerned with basic research". You go on to say that "specifically it should be possible to earn great benefits by incorporating many of the basic research units and stations maintained by the MRC and the ARC within universities and polytechnics". The idea of a close association between the research institutes and the universities is certainly not new and there has probably been as much movement in this direction in recent years as is possible when we realize that new buildings are often involved.

It is, however, a later remark in the editorial which I find much more disturbing: "But would not the merging of the research councils create too monolithic a central sponsor?" . . . "The case for welding them together into a more efficient (because larger) organization for supporting academic research is, first, that the funds available for such work will fall short of real need in the years ahead and that a properly organized and more powerful research council would be able to make more rational decisions about priorities in research."

We have heard many different ideas about the reorganization of the research councils in recent months, ideas which if acted upon would have a profound effect on the organization of research in this country. I am sure that this feeling of uncertainty has already done much harm to the efficient working of the councils. The scientists themselves feel as if they were being buffeted in a storm not knowing from which direction the wind will blow next. The idea of a monolithic research council really seems the most stupid of all the current ideas. It is easy to predict that no sooner had it been set up than it would be necessary to sectionalize it along the lines of the present research councils. The idea that size necessarily brings efficiency is extremely

naive. The Council for Scientific Policy should be capable of making rational decisions about priorities in research.

Yours faithfully,

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Kidney Transplants

SIR,—Your leading article "Nothing Much to Report on Transplants" (*Nature*, 231, 274; 1971) drew attention to the need for centres to coordinate the matching and distribution of cadaver kidneys so that patients needing renal transplants may receive as closely matched kidneys as possible. Your readers were given the impression that nothing is being done about this in Britain. In fact two centres have been working actively for the past two years, one in Newcastle and the other in London. Each provides a 24 hour service with a medically qualified immunologist backed up by a computerized register of recipient information. Each centre coordinates the typing and distribution of cadaveric kidneys to and from a group of collaborating hospitals. The centres in Newcastle and London work closely and amicably together and with the comparable organizations on the continent—Eurotransplant, Scandia-transplant and France-transplant.

The London Transplant Group began in 1968 as a collaboration between three hospitals: in 1970 there were 15: today there are 22. At first the pool of patients awaiting transplant was only 30: in 1970 it had risen to 170: today it stands at 400. By June 1970 the LTG had arranged 85 transplants: a year later this number had risen to 220.

By increasing the size of the recipient pool, it has been possible to improve the tissue matching. In 1969 less than one in ten transplants had as many as three of their four HL-A antigens in common. In the first 6 months of 1971, no less than 61% of all transplants arranged by the LTG had three or four HL-A antigens in common, whilst in sixteen transplants all four antigens were identical, a closeness of matching not yet approached by any similar organization in Europe or America.

The improvement in matching has been reflected in the survival of kidneys after

transplantation. If we exclude the kidneys which never survive the grafting operation, we find that 9 months later all those with 4/4 antigens in common are still functioning and 88% of those with 3/4 matches, whereas only 67% and 57% of those in two less well matched categories are still surviving. The differences between the best (3/4 and 4/4) and worst matches are highly significant.

We are not surprised that your leading article made no reference to either the Newcastle or the London centres. Neither of them gets any official approval or financial support from the Department of Health and Social Security. Quite the reverse: far from supporting either enterprise, the Department has recently decided to set up an entirely new centre at Bristol and destroy the existing organizations. The new centre, for which an expensive computer has been allocated, is to concern itself only with the British Isles. This is the reverse of progress. On the one hand we are likely to face a prolonged interregnum during which our patients will be deprived of well matched kidneys, since the necessary experience and know-how to run such a coordinating centre does not spring up overnight.

On the other hand, the cooperation with Europe and Eire which has been built up so laboriously, and which has already borne such fruit, is to be deliberately terminated. What is required now is not a new National Centre: like the existing regional network, it must surely be superseded by a European one. This should be the real object of the Department and, until it can achieve it, it would be grossly wasteful not to support existing centres in Newcastle and London.

Yours faithfully,

J. P. BLANDY
H. FESTENSTEIN

*The London Hospital,
Whitechapel,
London E1 1BB*

Scientologists

SIR,—I could scarcely believe my eyes when I read your Washington Correspondent's report on the US Food and Drug Administration's battle with the scientologists (*Nature*, 231, 485; 1971). The extreme vigour with which the FDA carried out its campaign against Wilhelm Reich might well be thought overdone,

though no one could seriously suggest (as your correspondent seems to allow) that sales of the orgone box could have continued.

It may just be that the scientologists are unjustly persecuted but all the evidence available suggests that they are a thousand times more dangerous to those who fall into their clutches than poor Reich ever was. The laughable scientific background to scientology does not prevent its being taken very seriously by some people.

It is foolish to say "the FDA alleges that the E-meters are a medical device". The scientologists themselves make claims tantamount to this. They may call themselves a church but your correspondent appears to be the first person to be taken in by this style.

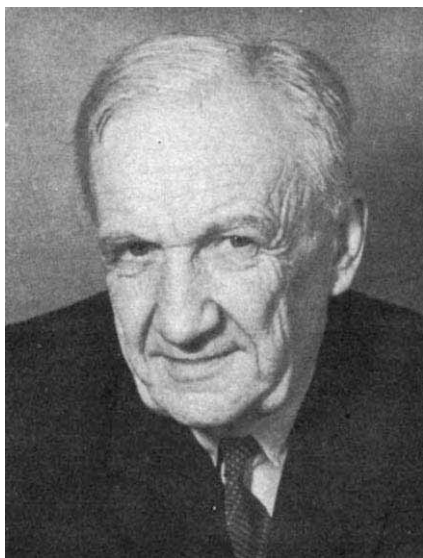
Yours faithfully,

CHRISTOPHER MACY

General Editor,
Rationalist Press Association Ltd,
88 Islington High Street,
London N1 8EW

Obituary

Professor I. E. Tamm



IGOR EVGEN'EVICH TAMM, who died on April 12, was one of the Soviet Union's most distinguished and best loved physicists. Born in 1895, the son of a civil engineer, he studied at Edinburgh from 1913, but at the outbreak of the First World War returned to Moscow University, where he graduated in 1918. At that time theoretical physics was not a highly developed subject in the Russian universities. Making this point in a biography of Ya. I. Frenkel, Tamm recalls that Maxwell's theory was deemed to be too complicated to be treated in lectures. "To be sure it was taught in a special

'elective' course by A. Bachinskii, but I was given a top grade in the examination on that course only because in deriving formulae on the blackboard I used the symbol for the vector product and knew its meaning; no other questions were asked of me at all."

This did not stop Tamm from getting thoroughly immersed in the subject, but at the outbreak of the Civil War academic life became very difficult, especially because he was teaching at this time in the University of the Crimea, a region particularly affected by the turmoil of the Civil War. The company of Frenkel, however, another young theoretical physicist with similar interests, was most valuable. 1921-22 was spent at the Odessa Polytechnic under L. I. Mandelstam, an outstanding physicist. This was the beginning of a lifelong close association.

In 1922 Tamm returned to Moscow University, where he became a professor in 1930. Later he joined the new Lebedev Institute of the Academy of Sciences, while remaining, most of the time, part-time professor at the university. His earliest research work was in the field of electrodynamics and relativity. When the new quantum mechanics developed in the late nineteen-twenties, Tamm picked up the new ideas with enthusiasm. He was one of the first to apply quantum mechanics to the scattering of light by solids, and thus to explain the discovery by Mandelstam and Landsberg of a doublet of lines in the scattered light. In another important piece of work on solids he showed the existence of surface states for electrons in metals.

When in 1934, Cherenkov, a research student in the same institute, noticed a strange radiation accompanying the passage of fast electrons through a transparent substance, Tamm saw at once that this was predicted by Maxwell's equations if the speed of the particles exceeded that of light in the medium. He worked out the full theory of this effect with I. M. Frank, and in 1958 shared the Nobel Prize with Frank and Cherenkov.

As time went on, his interest shifted more and more towards the fundamental problems in physics. He attempted to build a theory of nuclear forces on the phenomenon of β decay, but recognized that the resulting force would be much too weak. While this work therefore did not directly yield useful results, it aroused his interest in the treatment of interacting fields, and he later proposed a technique for approaching the theory of such problems which, under the name of "Tamm-Dancoff method", is still one of the important tools of the theoretical physicist. Numerous other papers dealt with more specific problems in the theory or with the interpretation of experiments.

During the war and post-war years he, like most other scientists, contributed to the solution of urgent practical problems.

Rumour says that he contributed to the development of the fusion bomb in the Soviet Union; he certainly did important work on the problem of power from fusion.

But his influence on physics transcends the list of specific achievements. He was a most stimulating influence in discussion, because of his clarity of mind, his speed in seeing the point and, above all, his infectious enthusiasm. He was a person of fearless integrity, direct and simple in manner, and of extreme modesty. From 1953 he was a full member of the Soviet Academy of Sciences, and thus in a position which confers such a high status and so many privileges that an average person may well be excused for taking himself a little seriously as a result. But for Tamm to display his own importance would have been quite unthinkable.

He was an enthusiastic supporter of the Pugwash conferences, of which he attended several, including the one in London in 1962, where he put forward the idea of "black boxes" to verify a nuclear test ban treaty. While this idea was not ultimately accepted in international negotiations, it helped to create a more constructive spirit and played its part in bringing about at least a partial test ban.

Tamm was a passionate mountaineer, of professional standard, and he spent much of his vacation time in the mountains until a few years ago. During his last illness, which kept him confined to bed with a breathing machine, he was not given to complaining, in spite of much discomfort, and he continued working on some new ideas, but he did admit his regret that this kept him from the mountains. He will be remembered not only as one of the most distinguished but also one of the most charming persons in the world of physics.

Errata

IN the article "Late Pre-Cambrian Glaciation in Australia as a Stratigraphic Boundary" by P. R. Dunn, B. P. Thomson and Kalervo Rankama (*Nature*, **231**, 498; 1971), the sentence starting on line 21 beneath the subheading Sturtian Glaciation as Boundary should read: "The base of the proposed unit is to be marked by a reference point, called the Sturt Marker (Marker Horizon), provisionally placed in South Australia at the base of the granite conglomerate which forms the bottom member of the Fitton Formation³³."

IN the article "Preliminary Observations on Tickling Oneself" by L. Weiskrantz, J. Elliott and C. Darlington (*Nature*, **230**, 598; 1971), the words "a self-administered" on line 17 of paragraph 6 should read "an externally administered".

International Meetings

August 2-3, **Brain-Endocrine Interaction: Median Eminence, Structure and Function**, Munich (Dr Karl M. Knigge, Department of Anatomy, University of Rochester, 260 Crittenden Boulevard, Rochester, New York 14642, USA).

August 2-3, **Physiology and Pharmacology of Synapses**, Basle (Dr L. Hoesli, Department of Neurophysiology, University of Basle, Socinstrasse 55, 4051 Basle, Switzerland).

August 2-3, **Information Transfer from a Neurophysiological and Neurochemical Point of View**, Helsinki (Dr M. Bergstroem, Institute of Physiology, University of Helsinki, Siltavuorenpenger 20A, Helsinki 17, Finland).

August 2-4, **Olfaction and Taste**, Starnberg (Dr K. E. Kaissling, Max-Planck Institute of Behavioural Physiology, 8131 Seewiesen/Starnberg, Germany).

August 2-4, **Comparative Physiology of Respiration in Vertebrates**, Göttingen (Dr J. Piiper, Max-Planck-Institute of Experimental Medicine, Hermann-Rein-Strasse 3, 34 Göttingen, Germany).

August 2-4, **Vision and Audition: Comparisons between the Systems**, Zillertal (Dr O. Creutzfeldt, Max-Planck-Institute of Psychiatry, Kraepelinstrasse 2/10, 8 Munich 23, Germany).

August 2-4, **The Somatosensory System**, Reisenburg near Ulm (Dr H. H. Kornhuber, Department of Neurology, University of Ulm, Steinhoevelstrasse 9, 79 Ulm, Germany).

August 2-5, **Renal Handling of Sodium**, Bristenberg (Dr F. Spinelli, J. R. Geigy AG, 4000 Basle 21, Switzerland).

August 2-5, **Invertebrate Neurology, Mechanisms of Rhythm Regulation**, Tihany (Dr J. Salanki, Biological Research Institute, Tihany, Hungary).

August 2-6, **Central and Peripheral Adrenergic Systems**, Warsaw (Dr A. Trzebski, Department of Physiology, Medical Faculty, Krakowskie Przedmiescie-26/28, Warsaw, Poland).

August 2-6, **Regulation of Food and Water Intake**, Cambridge (Dr J. T. Fitzsimons, Physiological Laboratory, Downing Street, Cambridge).

August 2-6, **Neural Control of Breathing**, Warsaw (Dr W. Karczewski, Neurophysiological Laboratory, Polish Academy of Sciences, Medical Research Centre, Dworkowa 3, Warsaw, Poland).

August 2-7, **Frontal Granular Cortex and Behaviour**, Jablonna near Warsaw (Dr J. Konorski, 3 Pasteur Street, Warsaw 22, Poland).

August 2-7, **Electric Field of the Heart**, Brussels (Dr P. Rijlant, Solvay Institute for Physiology, Medical Faculty of the University of Brussels, 115 Boulevard de Waterloo, Brussels 1000, Belgium).

August 2-8, **Mechanisms of Regulation of the Biogenic Amine Level in the Tissues with Special Reference to Histamine**, Lodz (Dr Cz. Maslinski, Department of

Physiopathology, School of Medicine, Narutowicza 60, Lodz, Poland).

August 3-4, **Slow and Fast Muscle**, Birmingham (Dr A. F. Huxley, Department of Physiology, University College, Gower Street, London WC1).

August 3-5, **Physical Fitness**, Prague (Dr V. Seliger, Department of Physiology FTVS, Ujezd 450, Prague, Czechoslovakia).

August 3-5, **Coupling between Electrolyte and Nonelectrolyte Transport in Cells**, Frankfurt (Dr E. Heinz, Institute of Physiology, Ludwig-Rehn-Strasse 14, 6 Frankfurt/Main, Germany).

August 3-7, **Biocybernetics**, Leipzig (Dr H. Drischel, Carl-Ludwig-Institute of Physiology, Leibigstrasse 27, 701 Leipzig, Germany).

August 3-7, **Neurohumoral and Metabolic Aspects of Injury**, Lodz (Dr Cz. Maslinski, Department of Physiopathology, School of Medicine, Narutowicza 60, Lodz, Poland).

August 4-6, **Gas Exchange across the Placenta**, Hanover (Dr W. Moll, Institute of Physiology, Roderbruchstrasse 101, 3 Hanover, Germany).

August 7-8, **Neural Control of Motor Performance**, Zurich (Dr K. Akert, Institute for Brain Research, University of Zurich, August Forel Strasse 1, 8008 Zurich, Switzerland).

September 4-7, **Molecular Genetics and Developmental Biology**, Woods Hole (Dr R. E. Stephens, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA).

September 8-11, **Education-Technology-Industry-Society**, Southampton (K. L. Morphew, Southern Science and Technology Forum, University of Southampton, Southampton SO9 5NH).

September 9-11, **Taxonomy and Phyto-geography of Higher Plants in Relation to Evolution**, Manchester (Miss J. Shore, Department of Botany, University, Manchester M13 9PL).

September 15-16, **Plastics in Medicine and Surgery**, Newcastle upon Tyne (A. J. Harrow, The Medical School, The University, Newcastle upon Tyne NE1 7RU).

September 16-17, **Explosion Hazards in the Chemical Industry**, Manchester (Registrar, University of Manchester Institute of Science and Technology, PO Box 88, Manchester M60 1QD).

September 16-17, **Insect-Plant Relationships**, London (The Registrar, Royal Entomological Society of London, 41 Queen's Gate, London SW7).

September 17-19, **Physics—from School through Higher Education**, Cardiff (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

September 25, **Computer Simulation of Laboratory Experiments**, London (The Secretary, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend on Sea, Essex SS1 2JY).

September 27, **Polymers in Water and Waste Treatment**, London (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1).

September 27-28, **Precipitates, Inclusions and Ordered Defects in Crystals**, Brighton (Professor C. H. L. Goodman, STL, London Road, Harlow, Essex).

September 28-30, **Information for the Construction Industry**, London (Education Department, Aslib, 3 Belgrave Square, London SW1X 8PL).

September 28-October 1, **Vertebrate Palaeontology and Comparative Anatomy**, Bristol (Mrs S. C. Savage, Department of Geology, Queen's Building, Bristol BS8 1TR).

September 29-30, **Microbial Control in Pharmaceutical and Cosmetic Preparations**, London (R. E. Marshall, Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London WC1A 2NN).

October 13-14, **Immunity and Cancer**, Paris (P. Burtin, Institut de Recherches Scientifiques sur le Cancer, BP8, 94 Villejuif, France).

December 6-8, **Ultrasonics**, Miami (Dr H. Matthews, Sperry Rand Research Center, 100 North Road, Sudbury, Massachusetts 01776, USA).

British Diary

Monday, July 12

An Introduction to the Digital Control of Noisy Systems (vacation school, four days) Institution of Electrical Engineers, at the University of Oxford.

Microwave Solid-state Devices (vacation school, twelve days) Institution of Electrical Engineers, at the University of Leeds.

Third Society for Analytical Chemistry Conference (five days) at the University of Durham.

Tuesday, July 13

Partial Differential Equations and Distributed Parameter Systems (four day conference) Institution of Electrical Engineers, in association with the University of Warwick, and the Institute of Mathematics and its Applications, at the University of Warwick.

Thursday, July 15

Comparative Physiology of Desert Animals (two day symposium) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

Monday, July 19

The Continuous Culture of Microorganisms (international symposium and study group, six days) Society of Chemical Industry, Microbiology Group, at St Catherine's College, Oxford.

Reports and Publications

not included in the monthly Books Supplement

Great Britain and Ireland

- Fish in Britain: Trends in Its Supply, Distribution and Consumption During the Past Two Centuries. Edited by T. C. Barker and John Yudkin. (Occasional Paper No. 2.) Pp. 90. (London: Department of Nutrition, Queen Elizabeth College, University of London, 1971.) £1 net. [65]
- Machinery's Buyers' Guide 1971. 42nd edition. Edited by Frederick A. J. Browne. Pp. 1478. (London: The Machinery Publishing Company Ltd., 1971.) £1.50. [65]
- Research Using Transplanted Tumours of Laboratory Animals—a Cross-Referenced Bibliography—VII. By D. C. Roberts. Pp. 170. (London: Imperial Cancer Research Fund, 1971.) [75]
- Celestial Physics Laboratories. By Sir Martin Ryle, FRS. (The John Coffin Memorial Lecture delivered before the University of London on May 21, 1970.) Pp. 19. (London: The Athlone Press, University of London, 1971.) 35p. [75]
- Preservation of Personal Health in Warm Climates. Seventh edition. Pp. 103. (London: The Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, 1971.) [75]
- The Wellcome Trust 1968-70: Eighth Report. Pp. 122. (London: The Wellcome Trust, 1971.) [75]
- British Flame Research Committee. 1970 Annual Report. Pp. 24. (London: British Flame Research Committee, 1971.) [115]
- British Industrial Biological Research Association. Annual Report 1970. Pp. 62. (Carshalton, Surrey: BIBRA, 1971.) [115]
- Fabian Research Series No. 293: People, Participation and Government. By a Fabian Group. Pp. 32. (London: Fabian Society, 1971.) 25p. [115]
- Royal Scottish Museum. A Catalogue of Fossil Vertebrates in the Royal Scottish Museum, Edinburgh. Part 1: Actinopterygii. By I. G. C. Henrichsen. Pp. 102. (Edinburgh: Royal Scottish Museum, 1970.) [115]
- Paterson Laboratories Annual Report 1970. Pp. 189. (Manchester: Christie Hospital and Holt Radium Institute, 1971.) [125]
- University of Sussex. Science Policy Research Unit—Fourth Annual Report. Pp. 46. (Brighton: University of Sussex, 1971.) [125]
- Causality and Determination. By Professor G. E. M. Anscombe. (Inaugural Lecture.) Pp. 30. (London: Cambridge University Press, 1971.) 30p. net; \$0.95 [125]
- Electronics at Essex. Pp. 39. (Colchester: Department of Electrical Engineering Science, University of Essex, 1971.) [125]
- The Heating and Ventilating Research Association. Annual Report 1970. Pp. 39. (Bracknell: The Heating and Ventilating Research Association, 1971.) [125]
- Proceedings of the Society for Psychical Research. Vol. 55, Part 202: First Sight—Second Sight. By Professor W. A. H. Rushton. (Presidential Address, 1970.) Pp. 177-188. (London: Society for Psychical Research, 1971.) [135]
- The Physics Teaching Handbook 1971. Edited by L. A. Redman. Pp. 232. (London: Longman Group Limited, Journals Division, 1971.) 75p. [135]
- Building Research Station Digest: Insulation Against External Noise—2. Pp. 4. (London: HMSO, 1971.) 5p. [135]
- Proceedings of the Royal Irish Academy. Vol. 71, Section A, No. 2: On the Rolling of One Curve or Surface Upon Another. By H. H. Hacisalihoglu. Pp. 13-17. (Dublin: Royal Irish Academy, 1971.) 10p. [145]
- Noxious Gases Underground: a Handbook for Colliery Managers. Pp. 33. (London: National Coal Board, 1971.) [145]
- Report of the Astronomer Royal for Scotland for the year ending 31 March 1971. Pp. 12. (London: Science Research Council, 1971.) [185]
- Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Volume XXVI, No. 7: Seasonal Movement of Young Plaice in the North-East Irish Sea. By H. W. Hill. Pp. iv+24. (London: HMSO, 1971.) 70p net. [185]

- The British Non-Ferrous Metals Research Association Annual Report 1970. Pp. 54. (London: British Non-Ferrous Metals Research Association, 1971.) [185]
- Gibraltar: Walks and Flowers. Pp. 35. (London: The Gibraltar Tourist Office, 15 Grand Buildings, Trafalgar Square, 1971.) 25p. [195]
- Albright and Wilson, Ltd., Annual Report 1970. Pp. 42. (London: Albright and Wilson, Ltd., 1971.) [195]
- Institution of Gas Engineers. Communications. No. 843: Computers, Communications and Customers. By K. E. Jones and W. V. Olsson. Pp. 24. £1. No. 844: Total Energy in the Gas Industry. By J. Pearson and P. B. Simpson. Pp. 10, 10p. No. 845: The Philosophy of Gas Storage. By D. J. Clarke, G. S. Brigg and W. J. Walters. Pp. 17. £1. No. 846: The Application of Work Study to Customer Service. By L. Ward. Pp. 10, 50p. No. 847: Natural Gas in Large-Volume and Industrial Markets (as Experienced by an Ontario Utility). By E. G. Moogk. Pp. 19. £1. No. 848: New Developments in Distribution in the United States. By Michael W. Anuskiewicz. Pp. 14. 50p. No. 849: The Development and Exploitation of Rapid Heating Techniques. By J. Masters, B. Oepen, C. J. Towler and P. F. Y. Wong. Pp. 18. £1. No. 850: Control System for the Gas Council's Transmission Network. By J. B. C. Bower, H. S. Jones, J. W. Smith and C. H. Townsend. Pp. 14. 50p. (London: Institution of Gas Engineers, 1971.) [195]
- Department of Trade and Industry. National Physical Laboratory—Measurement Group Research 1970. Pp. viii+147. (London: HMSO, 1971.) £1 net. [205]
- Proceedings of the Royal Irish Academy. Vol. 71, Section B, No. 6: The Freshwater Copepod Work of G. P. Farran together with Some Other Notes. By C. E. O'Riordan. Pp. 85-96. (Dublin: Royal Irish Academy, 1971.) 13p. [205]
- Units, Symbols and Abbreviations: a Guide for Biological and Medical Editors and Authors. Edited by George Ellis. Pp. 36. (London: The Royal Society of Medicine, 1971.) 54p. [205]
- The New University of Ulster. Report to the University Court 1968/1970. Pp. 49. (Coleraine: The New University of Ulster, 1971.) [205]
- British Museum (Natural History). Economic Leaflet No. 6: Plaster Beetles. Pp. 3. (London: British Museum (Natural History), 1971.) 3p. [215]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 259, No. 835, (20 May, 1971): The Response of Normal Men and Women to Changes in their Environmental Temperatures and Ways of Life. By R. A. McCance, H. E. Neil, N. E. Din, E. M. Widdowson, D. A. T. Southgate, R. Passmore, D. Shirling and R. T. Wilkinson. Pp. 533-565. (London: Royal Society, 1971.) £0.85; \$2.20. [215]

Other Countries

- Fisheries Research Board of Canada. Bulletin 173: Freshwater Fishes of Northwestern Canada and Alaska. By J. D. McPhail and C. C. Lindsey. Pp. 381. (Ottawa: Queen's Printer, 1970.) \$8.50. [204]
- An Introduction to the Choropteral Methods on Chemistry. By Pierre Crabbé. Pp. 121. (Mexico, DF: Mr. P. de Aguinaco, Apartado Postal 10-820, 1971.) \$4. [204]
- East African Community. East African Institute for Medical Research Annual Report, 1969. Pp. 63. (Mwanza, Tanzania: East African Institute for Medical Research, East African Community, 1969.) 25p. [204]
- Institut für Atomenergi. Kjeller Report 141: Optec—an Automatic Synthesis Program Package for Control of Dynamic Systems. By T. Johannessen. Pp. 23. (Kjeller, Norway: Institut für Atomenergi, Kjeller Research Establishment, 1971.) [204]
- Research and Studies, Carleton University, Ottawa. Pp. 223. (Ottawa: Carleton University, 1970.) [204]
- Canada: Department of Energy, Mines and Resources. Paper 70-B: Notes to Accompany Geological Maps of Antigonish and Cape George Map-Areas, Nova Scotia. By D. G. Benson. Pp. 2+2 maps. \$1.50 Paper 70-9: Notes to Accompany Maps of the Geology of Merigomish and Malignant Cove Map-Areas, Nova Scotia. By D. G. Benson. Pp. 3+2 maps. \$1.50. Paper 70-22: Marine Cretaceous Biotic Provinces and Paleogeography of Western and Arctic

- Canada—Illustrated by a Detailed Study of Ammonites. By J. A. Jeletzky. Pp. v+92. \$2. Paper 70-27: Eskimo Point and Dawson Inlet Map Areas (North Halves), District of Keewatin. 55E and 55F (north parts). By A. Davidson. Pp. 21. \$2. Paper 70-29: Geology of Berens River-Deer Lake Map-Area, Manitoba and Ontario and a Preliminary Analysis of Tectonic Variations in the Area. By I. F. Ermanovics. Pp. 24. \$1.50. Paper 70-41: Douglas Channel-Hecate Strait Map-Area, British Columbia. By J. A. Roddick. Pp. 556. \$2. Paper 70-47: Geological Exploration in the Coppermine River Area, Northwest Territories. By R. I. Thorpe. Pp. 150. \$2. Paper 70-59: A Miniaturized Digital Data Acquisition System for High Resolution Magnetometer Surveying. By P. Sawatzky. Pp. 95. \$2. (Ottawa: Queen's Printer, 1970.) [204]

- US Department of the Interior: Geological Survey. Professional Paper 644-C: Kaiser Peak Quadrangle, Central Sierra Nevada, California—Analytic Data. By Paul C. Bateman and John P. Lockwood. Pp. iii+15. \$0.30. Professional Paper 672: Geology of the Renton, Auburn, and Black Diamond Quadrangles, King County, Washington. By Donald R. Mullineaux. Pp. iv+92. \$1. Professional Paper 700-D: Geological Survey Research 1970, Chapter D. Pp. 317. \$3.75. Professional Paper 529-H: Atlantic Continental Shelf and Slope of the United States—Gravels of the Northeastern Part. By John Schlee and Richard M. Pratt. Pp. iii+39. Professional Paper 650-B: Geological Survey Research 1969, Chapter B. Pp. 201. \$2.75. (Washington, DC: Government Printing Office, 1969 and 1970.) [204]

- Catalogue of the Birds Eggs in the collection of the National Museums of Rhodesia. By H. W. James. Pp. x+237. (Salisbury: National Museums of Rhodesia, 1970.) [204]

- US Department of the Interior: Geological Survey. Water-Supply Paper 1893: Potential Development and Recharge of Ground Water in Mill Creek Valley, Butler and Hamilton Counties, Ohio. Based on Analog Model Analysis. By Richard E. Fidler. Pp. iv+37+4 plates. \$1. Water-Supply Paper 1899-A: Ground-Water Resources of the Clatsop Plains Sand-Dune Area, Clatsop County, Oregon. By F. J. Frank. Pp. iv+41+1 plate. Professional Paper 681: Fish Creek Mountains Tuff and Volcanic Center, Lander County, Nevada. By Edwin H. McKee. Pp. iii+17. \$0.35. (Washington, DC: Government Printing Office, 1970.) [214]

- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Land Research 1969-1970. Pp. 125. (Canberra: CSIRO, 1971.) [214]

- Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIII, No. 13: The Radial Velocities and Luminosities of 77 Stars in the Field of the Alpha Persei Cluster. By R. M. Petrie and J. F. Heard. Pp. 329-346. Vol. XIII, No. 14: Spectrographic Observations of Nova Delphini, 1967-1968. By J. B. Hutchings. Pp. 347-382. Vol. XIII, No. 15: A Further Study of the Triple System H.D. 10018 (A.D.S. 8189). By R. M. Petrie and A. H. Batten. Pp. 383-396. Vol. XIII, No. 16: An Analysis of Nova Delphini 1967. By J. B. Hutchings. Pp. 397-414. (Victoria, BC: Dominion Astrophysical Observatory, 1970.) [214]

- Mitteilungen der Prähistorischen Kommission der Österreichischen Akademie der Wissenschaften. XIII und XIV Band: Das Problem der Chronologie Jungpaläolithischer Stationen im Bereiche der Europäischen USSR. Von Erwin Lucius. Pp. 130 mit 78 tafeln, 5 tabellen und 1 karte. (Graz, Wien und Köln: Hermann Böhlau Nachfolger, 1969-1970.) [214]

- The R/V Pillsbury Deep-Sea Biological Expedition to the Gulf of Guinea, 1964-1965. (Studies in Tropical Oceanography, No. 4, Part 2.) Pp. viii+241+466. (Miami, Florida: University of Miami Press, 1970. Published for Rosenthal School of Marine and Atmospheric Sciences.) [224]

- University of Science and Technology, Kumasi. Annual Report 1968-1969. Pp. 130. (Kumasi: University of Science and Technology, 1971.) [224]

- The Second Fifty Years. By Dr. J. Melville. (Thomas Cawthron Memorial Lecture, No. 43.) Pp. 21. (Nelson, New Zealand: Cawthron Institute, 1970.) [224]

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